Digestion and Digestive Enzymes in the Herring (*Clupea harengus* L.)

By Helen I. Battle
University of Western Ontario

(*Received for publication September 8, 1934*)

ABSTRACT

Crustacean food is partially broken down and digested in the caecum of the stomach of the herring. It becomes more finely divided in the pyloric sac and consists of an oily chyme, intermingled with chitin, mucus and bacterial clumps in the pyloric caeca and intestine. The acidic condition of the gastric contents is probably instrumental in the reddening of chitinous food in the tract. Pepsin from the stomach and trypsin from the pyloric caeca increase in digestive power over a temperature range from 2.4 to 37.5°C. The stomach secretes a protease (pepsin), a weak amylase, and possibly a weak lipase. The pyloric caeca secrete a strong protease (trypsin), a strong amylase, and a lipase rendered active by bile. The intestinal mucosa exhibits lipolytic and amylolytic ferments, while the bile has some amylolytic properties.

INTRODUCTION

The present study is a summary of observations on certain characteristic features of the digestive organs and digestive processes in the immature herring (*Clupea harengus* L.), the sardine of commerce of the Canadian Atlantic coast. The data were collected during the summer of 1933 at the Atlantic Biological Station, St. Andrews, N.B., throughout and incident to the course of an investigation into the "clearing" rates of the digestive tract at various temperatures.

Herring decomposition is negligible before canning because the interval between removal from the weir and the steaming process is of short duration. Occasionally the digestive tracts of the fish are distended with food when they are removed from the water. These so-called "feedy" fish decompose rapidly and are entirely unsuitable for canning, since the abdominal wall becomes softened and ruptures under the lightest pressure. This softening is apparently due to the hypersecretion of digestive enzymes (Almy 1926). Enzymic activity of the digestive tract of the herring has already been rather thoroughly studied, notably by Stirling (1884) and Almy (1926).

On this basis the following account is given merely to shed some additional light on the nature of the digestive organs, the degree of digestion of food organisms at different levels in the tract and some further characteristics of the digestive ferments. It has served to indicate as well the advisability of further investigations along this particular line.

MORPHOLOGY OF DIGESTIVE TRACT

The alimentary canal of the herring (Huxley 1881, Stirling 1884) is comparatively simple in type and presents a short oesophagus, a Y-shaped stomach and a straight intestine into the anterior end of which open 18 to 25 thread-like pyloric caeca, and the bile duct (see figure 1*a*, 1*b*). The liver consists of a single lobe, within the dorsal surface of which the gall bladder is imbedded. No special microscopic examination was made for pancreatic tissue, but it was assumed to be a diffuse whitish mass located in the fatty tissue of the intestinal mesentery just anterior to the spleen.

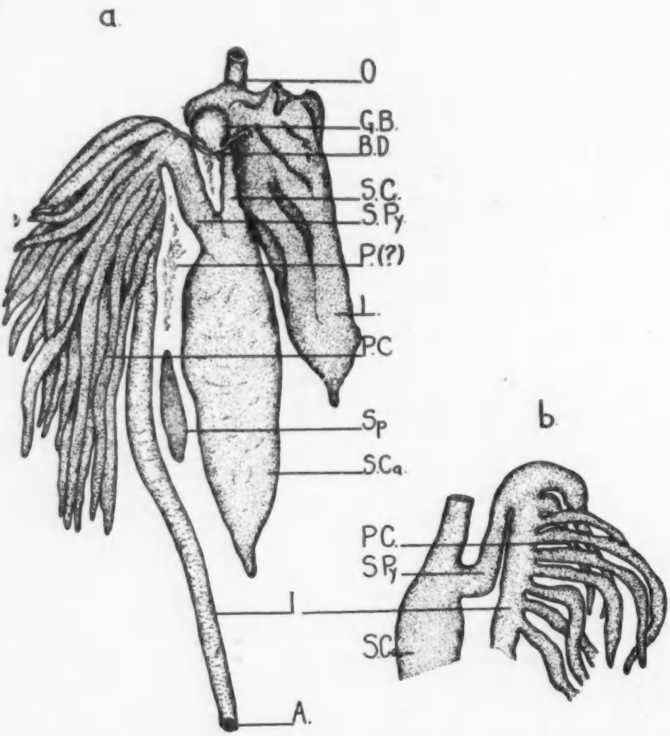


FIGURE 1. *a*—Digestive tract of herring dissected free from adipose tissue. A., anus; B.D., bile duct; G.B., gall bladder; I., intestine; L., liver; O., oesophagus; P., pancreatic tissue(?); P.C., pyloric caeca; S.C., cardiac division of stomach; S.Ca., caecum of stomach; S.Py pyloric division of stomach; Sp., spleen. *b*—Enlarged view of an area showing the entrance of pyloric caeca into intestine.

Huxley (1881) divided the stomach into two portions, the cardiac sac or "crop" and the muscular-walled pyloric sac or "gizzard". Not infrequently a constriction occurs about the middle of the tube leading from the pharynx to

the main portion of the stomach. It has seemed desirable to describe the portion anterior to this constriction as oesophagus, the posterior portion the *cardia* of the stomach (cardiac division), and the large blind tubular sac the *caecum* of the stomach. The *pyloric sac* (pyloric division) branches off anteriorly to the right from the junctional area of the cardia and caecum.

DIGESTION OF THE FOOD

The food of the herring consists largely of Crustacea and Chaetognatha. The former possess a high percentage of chitin which is comparatively indigestible and passes out apparently unchanged in the faeces. The general form of the stomach might lead to a supposition that the caecum is a blind storage organ, where digestion is probably of minor importance. Observations have been made on the food of 14 to 16 cm. herring at various levels in the digestive tract. Specimens were fed to gorging on copepods at 14°C. and five to seven hours later were fixed in formalin, after which the contents at various levels were examined microscopically.

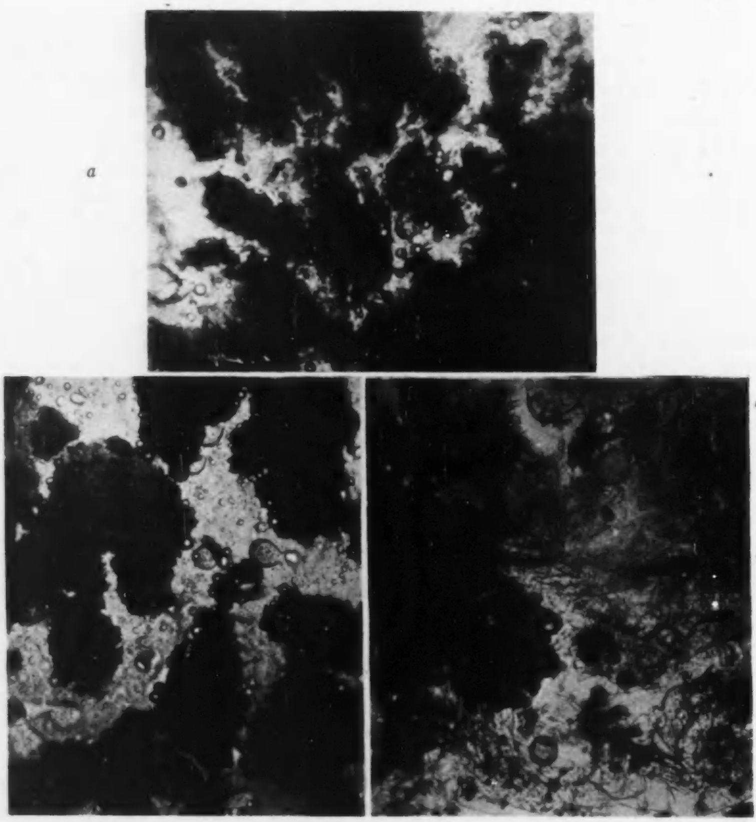
Copepods removed from the cardiac division of the stomach are normal, and unmutilated except for the loss of antennae or limb segments, which probably occurred as they were captured by the fish. In the caecum of the stomach the intersegmental tissue is partially broken down by the digestive ferments, resulting in minor fragmentation of the copepods. There are indications as well that there is some digestion of the musculature, and a great deal of the oil content of the animals has been freed as yellow to reddish globules. Microscopically the food in the pyloric sac (figure 2*a*) is seen to consist of finely divided portions of copepods, much of the chitin being entirely free from the fleshy remains of the animals. Figure 2*b* is a photomicrograph of the oily chyme found in the pyloric caeca six hours after feeding on a diet of copepods. The pyloric caeca are pale cream-coloured contracted structures in non-feedy fish, while in feedy specimens they appear enlarged and orange to reddish in colour due to the presence of oil and minute particles of partially digested crustacea. The functions of the pyloric caeca have long been debated. Bondovy (1899) considered that the absorptive role of "pylorique tubes" is a minimum, since food penetrates in very small quantities and the abundant mucus they secrete assists the passage of chyme in the intestine. However, Greene (1913) showed that they are the chief areas for fat absorption in the king salmon. Almy (1926) stated that in the herring "some at least of the liquid food residues leaving the stomach enter the ceca, undergo further digestion and are probably absorbed there."

Figure 2*c* shows the oily, chitinous and bacterial content of the intestine intermingled with mucous strands, some seven hours after feeding.

Herring excreta thus appear to possess a high percentage of oil as might be expected from the oily nature of the food. Corroborative evidence as to the oily nature of the faeces is given by the presence of "slick" on the water about the weir where "feedy" fish are clearing and also by the presence of oily excreta on the nearby shore-line at low tide.

Crustacean food on entering the stomach of a herring rapidly acquires a pinkish to reddish orange coloration which Almy (1926) attributes to the presence of chitinous tissue. It was found possible to produce this pinkish coloration by various methods. Live copepods were placed in various extracts of digestive juices, acids and alkalis as follows:

a



b *c*

FIGURE 2. *a*—Food removed from pyloric division of stomach of a 16 cm. herring, five hours after feeding. Note the disintegrated condition of the food, chitinous debris, etc. *b*—Contents of pyloric caeca of 16 cm. herring, six hours after a feeding of copepods. Observe the masses of oil globules in the chyme. *c*—Intestinal contents removed from a region posterior to the entrance of the pyloric caeca of a 16 cm. herring, seven hours after feeding. Note the chitinous remains, mass of oil globules, bacterial clumps, etc.

Copepods—*Eurytemora, Tortanus*

Solution	Time for production of complete pink coloration
0.36% HCl	7 min.
Gastric mucosa extract, pH. 2.6	15 min.
Pyloric caeca extract, pH. 9.0 ... still white in	24 hr.
0.36% CH_3COOH	14 min.
0.36% HNO_3	9 min.
0.36% KOH	12 hr.

There is the possibility that acidic conditions set up chemical reactions which cause the pink coloration, since alkalis seem to retard or inhibit its production. Unpreserved copepods remaining overnight in a small quantity of seawater become pink, perhaps because autolytic processes result in the production of acids. The same is true in formalin where free formic acid is probably present. In alkaline solutions the pink coloration may only appear when the autolytic processes have produced sufficient acid for neutralization.

When live *Meganyctiphanes* specimens are placed in extracts of herring digestive juices, they show certain changes as the death process ensues. In a distilled water extract of the gastric mucosa general body movements of *Meganyctiphanes* ceased in three minutes, while the appendages became motionless in another minute. Areas of opaqueness and pink coloration appear as indicated by stippling in figure 3. As might be expected, the tissues which most evidently are killed first are the gills, the intersegmental areas, the bases of the appendages, and the tissue in an area ventral to the eye in the region of the cephalic ganglion.

In *Calanus* and *Temora* similarly treated, movements cease in thirty seconds except for frantic inadequate twitchings of the limbs. Opaqueness (figure 3) commencing dorsally at the junction of the metasome and the posterior segments proceeds in V-shaped areas between the segments and rapidly takes in the whole organism.

DIGESTIVE ENZYMES

Digestive enzymes of the herring were first studied by Stirling (1884) and later by Almy (1926).

Stirling found that a very active pepsin was secreted by the "cardiac sac" (caecum) and could be extracted by "glycerine, dilute spirit, chloroform water and a solution of HCl (2 per 1000)" and was more active at 37 to 40°C. than at room temperature. A feebler pepsin was present in the pyloric sac. He also noted that the pyloric caeca when extracted with glycerine, 1 per cent Na_2CO_3 or alcohol yielded an extract with tryptic and diastatic properties, the latter property also being possessed by the bile.

Instead of always using fibrin as a substrate, Stirling washed the stomach contents of a herring possessing food, and neutralizing the partially digested contents (Crustacea or *Sagitta*), he found that they were rapidly digested.

PREPARATION OF EXTRACTS

Extracts of gastric mucosa were prepared by ligating the pyloric and cardiac limbs of the stomach and removing it from the body. It was cleared from mesentery and surrounding adipose tissue, split and washed several times in tap water. The mucosa was removed by scraping and ground with glass or sea sand in a mortar. Extracts were made with 30 per cent alcohol, glycerine, distilled

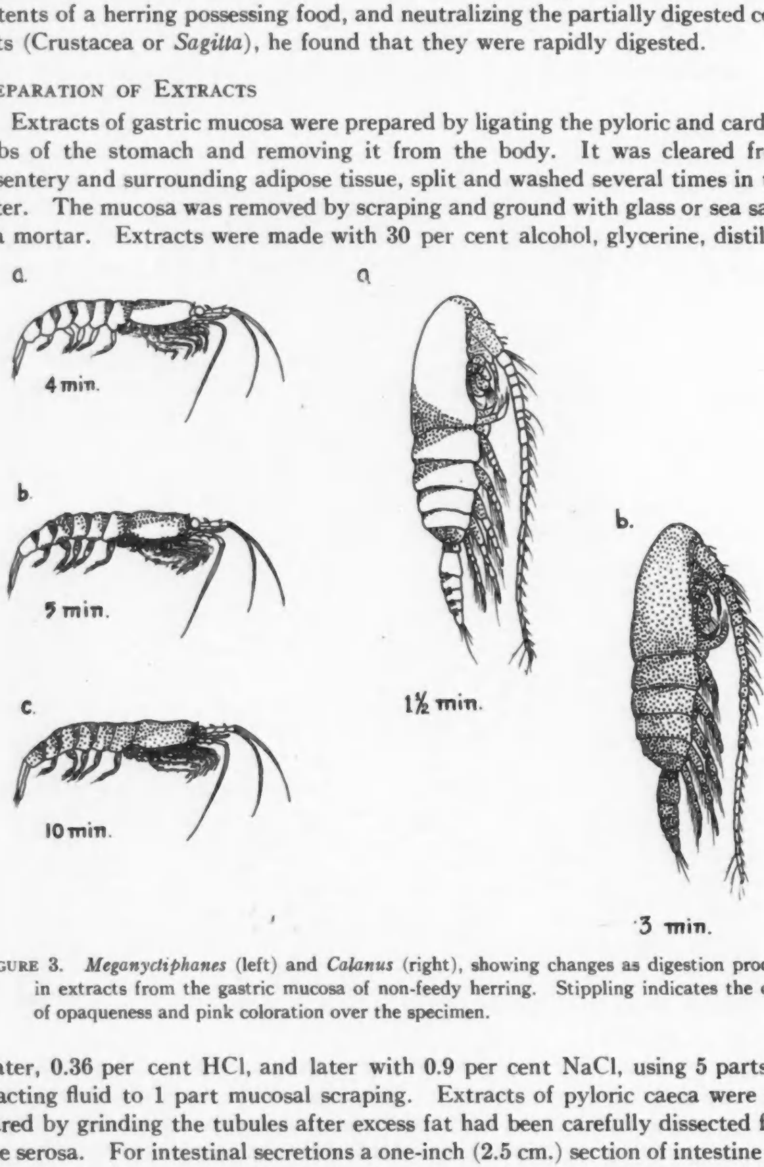


FIGURE 3. *Meganyctiphanes* (left) and *Calanus* (right), showing changes as digestion proceeds in extracts from the gastric mucosa of non-feedy herring. Stippling indicates the onset of opaqueness and pink coloration over the specimen.

water, 0.36 per cent HCl, and later with 0.9 per cent NaCl, using 5 parts extracting fluid to 1 part mucosal scraping. Extracts of pyloric caeca were prepared by grinding the tubules after excess fat had been carefully dissected from the serosa. For intestinal secretions a one-inch (2.5 cm.) section of intestine just posterior to the entrance of the pyloric caeca was ligated at either end and freed from mesentery, adipose and pancreatic tissue. It was then opened longitudinally and the contents were thoroughly washed before the mucosa was removed

and treated as the gastric mucosa had been. Extracts of liver were prepared by grinding it after removal of all fat. Bile was removed from the gall bladder by a fine sterile pipette.

Extraction was carried on at 2 to 3°C. by intermittent mechanical shaking for from 4 to 24 hours. The mixture was filtered in the cold and the pH adjusted to 2.6 for gastric extracts by addition of 0.36 per cent HCl and to appromimately 9.0 for pyloric caecal extracts by adding dry Na_2CO_3.

Table I shows the relative efficiency of various extracts in digesting the fresh Euphausiid, *Meganyctiphanes norvegica*. The living shrimps were wiped dry of moisture with filter paper, weighed and placed in 5 cc. of distilled water and 1 cc. of extract. At the end of 19½ hours they were removed, dried as previously and reweighed. The percentage loss in weight was taken as percentage digestion. A number of experiments proved the efficiency of the distilled water extracts and the technique of Almy (1926) was followed where the enzyme extract filtrate was diluted with an equal volume of glycerol to act as an enzyme preservative. The caecal extract was also diluted with four volumes of 1:1 glycerol solution before testing to obtain a rate curve comparable with that for the stomach extract.

TABLE I. Relative efficiency of various extracts in digesting the fresh Euphausiid, *Meganyctiphanes norvegica*

Temperature—37.5°C. Time—19 1/2 hours

Enzyme	Extracting medium	pH	% digestion
Caecum of stomach (pepsin)	Glycerine	2.6	55.1
" " " "	0.36 HCl	2.6	71.8
" " " "	Distilled water	2.6	67.1
Dog (pepsin)	Diluted 1:5	2.6	80.4
Pyloric caeca (trypsin)	Glycerine	9.0	56.5
" " "	0.36 HCl	9.0	52.0
" " "	Distilled water	9.0	75.8

TYPES OF SUBSTRATE

It was not possible to have available at all times a supply of live copepods or shrimps as a substrate for the criterion of enzyme activity, so that various methods of preservation were studied. From the results given in table II, showing the action of pyloric caecal extract on *Meganyctiphanes*, it would seem that either rapidly frozen (at −17°C.) or glycerine-preserved specimens, which had been thoroughly washed in running water for an hour previous to weighing, were quite as suitable as the fresh shrimp.

TABLE II. Relative efficiency of distilled water extracts from pyloric caeca on crustacean food, fresh and preserved

Temperature—37.5°C. Time—12 hours
pH 9

Substrate	Preservation	% digestion
Calanus	Frozen	40.4
Meganyctiphanes	Frozen	47.7
	Glycerine	48.7
	Fresh (alive)	50.5
	Formalin	3.1

The problem of determining even roughly the percentage digestion of *Calanus* was a difficult one. The following method was chosen. Five samples of copepods (approximately 0.5 gm. each) were weighed in the frozen condition, excess moisture having been removed before freezing. These were dried to constant weight in an oven at 90°C. and the loss in weight was found to be approximately 80 per cent. For experiments, known weights of copepods were allowed to be acted on by digestive ferment extracts for definite periods. These were then filtered, dried to constant weight and the percentage digestion calculated on the original basis of 80 per cent H_2O and volatile substances in the substrate.

Mett's tubes were used in proteolytic experiments merely as a parallel guide to the animal substrate. Bodansky and Rose (1922) found that coagulated egg white is very slowly digested by proteolytic enzymes of the fish. However,

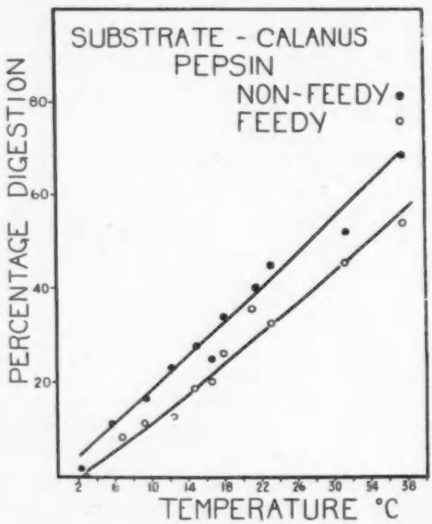


FIGURE 4. Relation between temperature and digestive activity of extracts from the gastric mucosa of feedy and non-feedy herring (substrate,—*Calanus finmarchicus*).

Kenyon (1925) states that peptic digestion of coagulated egg albumin per given weight of moist stomach mucosa is uniform for the representatives of all classes of vertebrates which he studied.

TEMPERATURE EFFECT ON DIGESTIVE ACTIVITY (PROTEOLYTIC)

Using *Calanus finmarchicus* and *Meganyctiphanes norvegica* as substrate, the relative digestive powers of gastric mucosal and pyloric caecal extracts of non-feedy and feedy fish were studied at various temperatures. In each instance 1 cc. of extract was used to 5 cc. of distilled water in which the substrate was placed upon weighing.

Table III, the results of which are represented graphically in figure 4, shows

that the gastric mucosal extract from non-feedy is more active on *Calanus* than that from feedy fish and that its activity rises with temperature, showing almost a straight line relationship from 2.4 to 37.5°C.

TABLE III. Percentage digestion of *Calanus finmarchicus* by gastric mucosal extract from non-feedy and feedy fish at various temperatures

pH 2.6					Time—22 hours
Extract	Non-feedy			Feedy	
substrate	*Calanus*	Metts		*Calanus*	Metts
Temperature(°C.)			Temperature		
2.4	2(?)	0	2.6	0	0
5.8	11.6	0	6.8	8.5	0
9.5	17.1	0	9.2	10.9	0
12.2	23.9	0	12.5	13.1	0
15.0	28.3	trace	14.6	19.6	0
16.6	25.7	trace	16.5	20.3	0
18.0	34.3	0.2	18.0	26.9	trace
21.4	40.7	0.4	21.0	35.3	0.1
23.0	45.6	0.8	23.0	33.5	0.3
31.4	53.3	1.7	31.5	46.0	1.2
37.5	69.9	4.0	37.5	54.5	2.3
37.5 (dog*)	86.8	20	37.5 (dog)	84.9	22

*Gastric juice diluted 1:5 with 0.36 HCl.

Table IV, with its graphic representation in figure 5, indicates that the pyloric caecal extract from feedy fish is more active on *Calanus* than that from non-feedy fish, and, as with the gastric extract, its activity increases with temperature, there being practically a straight line relationship from 2.4 to 37.5°C.

TABLE IV. Percentage digestion of *Calanus finmarchius* by pyloric caecal extract from non-feedy and feedy fish at various temperatures

pH 9.0					Time—22 hours
Extract	Non-feedy			Feedy	
substrate	*Calanus*	Metts		*Calanus*	Metts
Temperature(°C.)			Temperature		
2.4	6.2	0	2.6	5.8	0
5.8	8.8	0	6.8	32.7	trace
9.5	14.2	0	9.2	41.5	0.04
12.2	10.7	0	12.5	52.0	0.16
15.0	16.2	trace	14.6	50.3	0.16
16.6	21.3	trace	16.5	55.2	0.49
18.0	26.3	0.04	18.0	53.1	0.81
21.4	25.6	0.30	21.0	73.1	2.0
23.0	23.8	0.50	23.0	75.2	2.6
31.4	33.0	1.0	31.5	84.8	4.0
37.5	51.2	2.3	37.5	86.4	4.8

Table V and figure 6 represent the action on *Meganyctiphanes* of gastric and pyloric caecal extracts from non-feedy herring over the temperature range from 2.4 to 37.5°C. The results indicate, as shown by Kenyon (1925) and Almy (1926),

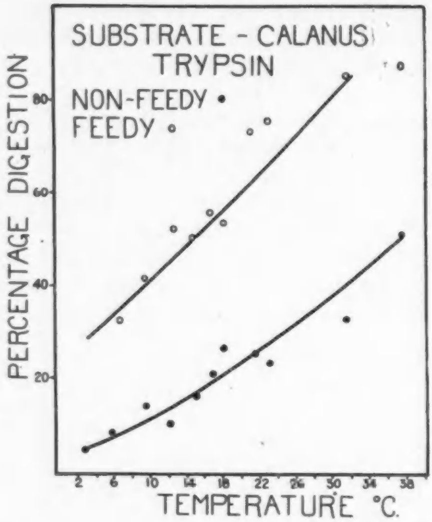


FIGURE 5. Relation between temperature and digestive activity of extracts from the pyloric caeca of feedy and non-feedy herring (substrate,—*Calanus finmarchicus*).

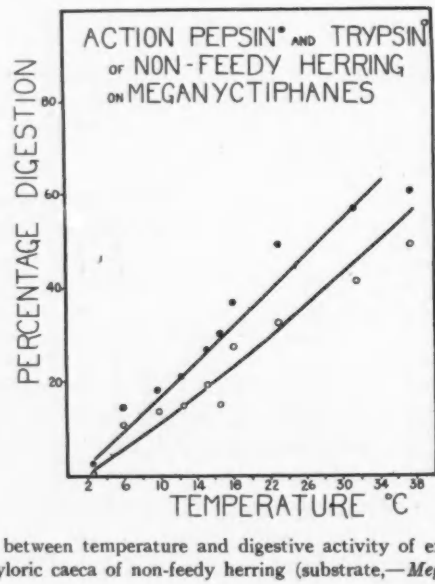


FIGURE 6. Relation between temperature and digestive activity of extracts from the gastric mucosa and pyloric caeca of non-feedy herring (substrate,—*Meganyctiphanes norvegica*).

that digestion is more rapid at 37°C. than at lower temperatures, and that more pepsin is extractable from non-feedy fish than from feedy, while the converse is true for the tryptic ferment of the pyloric caeca.

TABLE V. Percentage digestion of *Meganyctiphanes norvegica* by extracts of gastric mucosa and pyloric caeca of non-feedy herring at various temperatures

Time—22 hours

Temperature (°C.)	Gastric mucosa pH 2.6	Pyloric caeca pH 9.0
2.4	0.3(?)	0
5.8	14.2	10.7
9.5	18.1	13.9
12.2	21.4	14.3
15.0	27.1	19.3
16.6	31.7	15.1
18.0	36.4	27.2
21.4	31.8	21.3
23.0	49.6	32.6
31.4	56.8	41.8
37.5	60.4	49.7
37.5*	86.5	

*Dog pepsin diluted 1:5 with 0.36% HCl.

AMYLASE OF DIGESTIVE TRACT

Table VI gives the results of tests made for amylolytic activity in various parts of the digestive tract, using soluble starch as substrate, the iodine test for starch and dextrin, and Benedict's qualitative reagent for reducing sugar. Stirling (1884) found a diastase present in the pyloric caeca and bile of the herring, while Kenyon (1925) states that amylase is generally present in very small amount in the mucosa of the entire alimentary canal of fish. In the herring this would seem to be true, since it is apparently in the stomach, pyloric caeca, intestine, liver, and bile, though in comparatively large amount in the pyloric caeca.

TABLE VI. Amylolytic activity in aqueous extracts from various regions of the digestive tract of the herring

pH 6 Temperature—37.5°C.
Incubation period—24 hours
Substrate: 1% soluble starch
A 2 cc. starch+8 drops enzyme
B 2 cc. starch+8 drops enzyme (boiled)
C 2 cc. starch (control)
Tests: starch,—iodine; sugar,—Benedict's qualitative reagent

Extract	A Tests Starch	A Tests Sugar	B Tests Starch	B Tests Sugar
Stomach	+	+	+	−
Pyloric caeca	−	++	+	−
Intestine	reddish	++	+	−
Liver	+	+(slight)	+	−
Bile	+	+(slight)	+	−
C (control)	+	−		

Lipase of Digestive Tract

Table VII shows the lipolytic activity of various parts of the herring digestive tract. There appears to be a very weak lipase in the stomach but a fairly strong one in the intestine and pyloric caeca. Extracts of the latter seem to be accelerated in their activity by the bile. The presence of lipase in the pyloric caeca is generally denied (Bondovy 1899), but a weak form was found in the red snapper (*Lutjanus aya*) by Bodansky and Rose (1922). In the herring it would seem that the pyloric caeca do actually take over a considerable portion of digestive activity usually attributed to the pancreas.

Table VII. Lipolytic activity in aqueous extracts from various regions of the digestive tract of the herring

Substrate: 3 cc. boiled cream diluted 1:1 with distilled water.
Temperature—37.5°C. pH 8.3

Extract	No. of cc. of 0.09% NaOH required to bring back pink colour of phenolphthalein in 24 hours	
	Cream+5 drops extract	Boiled control
Cream (only)	....	0
Stomach	1.6	5 drops
Intestine	12.0	0
Pyloric caeca	5.1	0
Bile	4 drops	0
Bile+intestinal extract	16.4	3 drops
Bile+pyloric caecal extract	12.8	5 drops
Liver	1.0	0

Scale Digestion

Herring taken in weirs frequently contain many scales in the stomach. There has been some question as to their digestibility and why they are taken in at all. It is possible that, in seining, the fish are excitable, and while gulping air and water accidentally acquire the scales which have been brushed off by the seine; or that they are attracted by the iridescent appearance of the scales and actually swallow them voluntarily. If the latter be true it might seem that there is a possibility of digestion taking place. On September 7th approximately 100 scales from a freshly killed herring were washed carefully and placed in each of two test tubes containing: (*a*) extract of gastric mucosa pH 2.6; (*b*) extract of pyloric caeca pH 9.0.

The scales in the former became very transparent in a few hours and when later observed on November 15th had entirely disappeared macroscopically, while those in the pyloric caecal extract were quite intact, the pH having been modified to 2.8 and 7.8 respectively. The gastric extract had apparently removed the hyalodentine of the scale and only the microscopic traces of the inner fibrous layer with scattered iridescent particles remained.

Both extracts were still active on fibrin in December, 1933, and March, 1934, so experiments were repeated. It was found that 0.36 HCl acid alone will render

scales transparent and dissolve them in about seven weeks, whereas herring pepsin at a pH of 2.6 accomplishes the same results in approximately a week less time. Trypsin from the pyloric caeca was again found to be incapable of breaking up the scales. These experiments are not conclusive by any means and would merit a careful repetition.

CONCLUSIONS AND SUMMARY

A study of the contents of the digestive tract of the herring was made at various intervals following feeding on a typically crustacean diet of copepods.

The food is partially broken down and digested in the caecum of the stomach, becomes more finely divided in the pyloric sac and is found to consist of a viscid oily chyme mixed with chitin and clumps of bacteria in the pyloric caeca and intestine. Chitin is not digested but passes out with the faeces.

Acid and acidic extracts of the gastric juice produce a reddening of typical organisms with a chitinous exoskeleton. It may be concluded that the acidic gastric contents are instrumental in the reddening of food (popularly known as "red-feed" by fishermen) shortly after it enters the tract.

Using as a substrate typical food of the herring, namely, *Calanus* and *Meganyctiphanes*, the results of Almy (1926) are corroborated, namely, that pepsin from the stomach and trypsin from the pyloric caeca increase in digestive power with a rise in temperature, which ranged in these experiments from 2.4° to 37.5°C.; and also that less pepsin can be extracted from feedy fish than from non-feedy fish, while the reverse is true for extraction of trypsin from the pyloric caeca.

Experiments indicate the presence of weak lipolytic and amylolytic ferments in the gastric juice, but strong ones in the pyloric caecal and intestinal mucosal extracts. The lipase in the latter instances is rendered more active by bile.

The stomach of herring taken in weirs may be gorged with fish scales. The acidic gastric juice can remove the hyalodentine from these scales, leaving only microscopic traces of the inner fibrous layer, but the basic extract from the pyloric caeca has no effect on the scales, even over a period of months.

ACKNOWLEDGMENTS

My thanks are due to the Biological Board of Canada for use of the facilities of the Atlantic Biological station, St. Andrews, N.B., to Dr. A. G. Huntsman and to Dr. B. P. Babkin for advice and valuable assistance received in the course of the study.

REFERENCES

Almy, L. H. *J. Amer. Chem. Soc.* **48**, 2136-2146. 1926.
Bodansky, M. and W. C. Rose. *Amer. J. Physiol.* **62**, 452-487. 1922.
Bondovy, Th. *Arch. Zool. Exper.* **27** (Ser. 3) **T7**, 419-460. 1899.
Greene, C. W. *Bull. U.S. Bur. Fish.* **33**, 153. 1913.
Huxley, T. *Nature*, **23**, 607-613. 1881.
Kenyon, W. A. *Bull. U.S. Bur. Fish.* **41**, 181-200. 1925.
Stirling, Wm. *J. Anat. Physiol.* **18**, 426-435. 1884.

Ages at Migration of Atlantic Salmon (*Salmo salar*) in Miramichi River

By A. A. Blair
Atlantic Biological Station
(*Received for publication May 17, 1934*)

ABSTRACT

In 1,557 fish examined, the groups, based upon years in the sea and spawning marks, were:—grilse or 1+, 2 and 2+, 3 and 3+, and 1 S.M. and 2 S.M. groups. The 2 and 2+ form 85.1 per cent of the total (grilse excluded), and S.M. 12.8 per cent, and the 3 and 3+, 2.1 per cent.

Of fish with one spawning mark, 68.3 per cent first spawned as grilse, 29.9 per cent after two sea winters, and 1.8 per cent after three sea winters. The S.M. group had 11 per cent return to river in less than a year after first spawning, the remainder in little better than a year. Most of the fish with two spawning marks had the same length of absence on both occasions.

Only four smolt ages were found:—3 (78.1 per cent), 2 (15.1 per cent), 4 (6.6 per cent) and 5 (0.2 per cent).

INTRODUCTION

This paper deals particularly with the salmon from one river, the Miramichi, but attempts to show the variations occurring in other rivers of eastern Canada. We are here concerned only with the ages of the smolts or young salmon when leaving the river, and the ages of the older salmon when returning from the sea to the river for spawning purposes. Their ages have been determined by the usual method of scale reading. The results are based on material collected from the estuary and bay of the Miramichi river system in 1931.

The fish from the estuary were obtained as follows:—271 salmon from the fish house of Mr. A. F. Betts at Millerton from June 2 to August 29; 421 salmon and 46 grilse from the A. and R. Loggie Company at Loggieville from June 10 to October 9; 88 salmon and 128 grilse from Mr. David Kelly's salmon stand at Loggieville from July 2 to September 16. The fish from the bay consisted of 603 salmon obtained from the W. S. Loggie Company at Hardwicke from June 6 to September 17, and were caught eastward of a straight line extending from Escuminac breakwater to the lighthouse at the western end of Neguac island, but usually not more than 12 miles out.

SEA AGES

Under the heading of sea ages, the fish have been grouped according to the number of winters passed in the sea, regardless of their actual age or the number of years spent in the river. In addition, each of the above groups has been divided into one group containing fish which have not grown since the preceding winter, and another group composed of fish which have added some summer

growth during the year of capture. Also, fish which have previously spawned have been treated in a separate group regardless of age.

Age Composition of Total Stock

The fish from the Miramichi river may be separated into six such groups. The youngest sea group is composed of grilse (1+), i.e., fish which have passed one winter and part of the succeeding summer in the sea. The next older group (2) has remained two winters in the sea, but has not grown any for the current summer. Then we have the 2+ group which has passed two winters in the sea and part of the next summer. The 3 group has remained three winters only in the sea, while the 3+ group has spent part of the next summer in addition. Finally, we have the group S.M. composed of previously spawned fish which have spawned on one or two occasions.

As the grilse are not properly represented in the total sample which we are discussing, since they are too small to be caught in the drift nets, it will be necessary to consider the catch of salmon and grilse in one of the stands in the river. For this purpose a stand at Loggieville, seventeen miles from the mouth of the estuary, has been chosen. Fishing in 1931 began on May 15, and table I shows the weekly catch (per cent of total) of salmon and grilse for this particular stand.

Table 1. Weekly catch (per cent of total for season) of grilse and salmon in a trap-net at Loggieville, 1931

Week ending	Percentage of grilse	Percentage of salmon	Week ending	Percentage of grilse	Percentage of salmon
May 23	....	8.0	July 18	7.2	3.7
30	....	8.5	25	6.5	3.2
June 6	0.7	9.0	Aug. 1	5.5	6.4
13	4.8	10.1	8	8.9	16.0
20	22.1	11.2	15	1.0	6.4
27	22.1	6.9	22	1.7	3.2
July 4	11.3	2.7	29	1.0	3.7
11	7.2	1.1			
Total no.	....	....		293	188

The salmon were about two weeks earlier in running than the grilse, which did not commence until June. Yet an early run of salmon around the first of May was reported and a few grilse were also taken in May in some stands. Nevertheless, the main run of grilse was somewhat later than that of salmon. The most grilse (44 per cent) were taken during the last two weeks in June. Thereafter, the catch gradually decreased to 5.5 per cent in the latter part of July, but the first week in August showed a slight increase to 8.9 per cent while the catch of salmon was also considerably better during the same week. Most of the

salmon were taken in May and June, especially the middle of June. Then the catch of salmon begins to drop off during the latter part of June, and July is a very poor month for both salmon and grilse.

It is interesting to note that the grilse make up 60.9 per cent of the total catch of salmon and grilse for the season in this stand. However, the proportion of grilse would not be so great if the grilse could also be caught in the drift nets. Nevertheless, this is in strong contrast to the rivers farther north such as the Cascapedia, where Calderwood (1927) reported an absence of grilse, and the Moisie river where only one grilse out of a total of 1,585 fish was found (Macfarlane 1928). The other extreme is reached in the Minas system where 87 per cent were grilse or had spawned as grilse (Huntsman 1931). So, in respect to the proportion of grilse and salmon in the catch, the Miramichi river seems to be intermediate between the rivers to the north and to the south of it.

In considering the relative importance of the remaining sea groups, a sample of 1,383 fish will be used, i.e., the total sample less the number of grilse (174). The numbers have been separated into successive half-monthly periods and the percentage of each sea group in the various periods is recorded in table II.

TABLE II. Percentages of sea groups in combined drift and river fish during successive half-monthly periods of the season of 1931

Winters in sea	2	2+	Total 2, 2+	3	3+	Total 3, 3+	1 S.M.	2 S.M.	Total S.M.	Total no.
June 1–15	20.4	69.3	89.7	3.6		3.6	5.8	0.7	6.5	137
16–30	12.9	76.3	89.2	1.0	1.0	2.0	8.8		8.8	295
July 1–15	7.3	83.0	90.3	1.2	0.6	1.8	7.3	0.6	7.9	165
16–31	9.8	74.9	84.7	0.9	1.4	2.3	13.0		13.0	215
Aug. 1–15	12.0	66.1	78.1	0.5		0.5	18.6	2.7	21.3	183
16–31	7.7	71.5	79.2				17.7	3.7	20.8	130
Sept. 1–15	2.5	79.8	82.3	1.7		1.7	15.1	0.8	15.9	119
16–30		83.5	83.5	2.9	1.0	3.9	11.7	1.0	12.7	103
Oct. 1–15	2.8	80.6	83.4	8.3		8.3	8.3		8.3	36
Total	9.8	75.3	85.1	1.5	0.6	2.1	11.9	0.9	12.8	1383

First of all it is quite evident that the bulk of the commercial fishery is dependent upon the 2 and 2+ sea groups, since they make up 85.1 per cent of the total, provided, of course, that our sample is representative of the catch. This is somewhat similar to the condition in the Saint John river where the 2+ sea group makes up 97.9 per cent of the total (Huntsman 1931).

Of next importance to the fishery are the previously spawned salmon which form 12.8 per cent of the total. This proportion is considerably higher than in

the rivers of the eastern coast of Scotland where they generally form 4 per cent of the total (Menzies 1925*a*). However, in comparison with other rivers of Canada this percentage is rather low. The highest percentage for a Canadian river is 35.1, reported by Calderwood (1927) for the Grand Cascapedia. The proportion for the Moisie river varied from 16.7 to 28.7 per cent, as recorded by Menzies (1925*b*) and Menzies and Macfarlane (1927).

The 3 and 3+ sea groups are very poorly represented, forming only 2.1 per cent of the total. In some of the more northern rivers they are the most important sea groups. Calderwood (1927) found that they formed 58.8 per cent of the total in the Cascapedia river. Likewise in the Moisie river these two groups make up 71.2 per cent of the total (Macfarlane 1928). In the Restigouche river the 3 and 3+ sea groups are not so important as in the Cascapedia and Moisie rivers, but still they differ considerably from the same groups in the Miramichi river, since Phelps and Belding (1930) found that they formed 41.3 per cent of the total.

Variations in Age Composition during Fishing Season

Perhaps it is appropriate here to mention that the "open season" for drifting in 1931 extended from June 1 to July 31, with an extension in September from the 7th to the 16th inclusive, while the "open season" for the stands in the river lasted from May 15 to August 31 with a "close season" during the first two weeks of July. The above mentioned extension applied to the river fishing also.

As one would expect, the percentage of the 2 group decreases from over 20 per cent in the first part of June to less than 3 per cent the first part of October. The percentage of the 2 group drops suddenly to 7.3 per cent in the first half of July at which period the catch consists mainly of drift fish since it is a "close season" for river fishing. The 2 group must at that period be relatively scarce in the drift fish. In the two months of June and July the proportion of the 2 group in the drift fish is 7 per cent, while it is 22.9 per cent in the river fish (table III). Also the 2+ group forms 82.1 per cent of the drift catch and only 63.9 per cent of the river catch. But why should the river fish contain more of the 2 group and fewer of the 2+ group in these two months than the drift fish? The explanation is that in the total catch of the drift and river fishery the numbers of fish in the 2 group are proportionately decreasing as the season advances and at the same time these fish are leaving the drift area and moving into and up the river. So that the relative number of fish in the 2 group in the river fishery is increasing at the expense of the drift fishery.

The population of salmon in the 1 S.M. group increases more or less with the advance of the season. The low percentage of this group in June and July is mainly due to the influence of the drift fishery, where it forms only 7.3 per cent of the total, while for the same months in the river fishery it forms 12.8 per cent of the total (table III). The sudden drop to 7.3 per cent in the first half of July would be due to the drift fish as similarly explained for the 2 group. In addition the short absence fish (table IV) form 11 per cent of the 1 S.M. group and prac-

tically all of these fish were caught after July. So these two factors combine to give a low percentage of fish with one spawning mark in June and July.

The up trend in the proportion of the 1 S.M. group in the total and the down trend in the proportion of the 2 group throughout the season are reflected in the various proportions of the 2+ group. As explained before, the remaining groups (3, 3+, 2 S.M.) are very poorly represented. The proportion of the 3 group in the total is much greater at the beginning and at the end of the season than during the summer months. The percentage of the 3 group in the drift and in the river fish at different periods during the year is as follows:—

	June 1-15	June 16-30	July 1-15	July 16-31	August 1-15	August 16-31	September 1-15	September 16-30	October 1-15
Drift	5.6	1.1	1.3	1.6	...	...	6.3	4.0	...
River	0.0	0.9	0.0	0.0	0.5	0.0	0.0	2.6	8.3

The percentage of the 3 group in the drift is considerably greater in early June and in September than it is in late June and July (no drifting during August and October). The percentage of the 3 group in the river was practically negligible until late September and October. So it is quite probable that the 3 group assemble in the drift area during spring and summer months, but do not attempt to ascend the river until spawning time in the fall.

TABLE III. Percentage of each sea group in drift and in river fish for June and July

Sea group	2	2+	Total 2, 2+	3	3+	Total 3, 3+	1 S.M.	2 S.M.	Total S.M.	Total no.
Drift fish	7.0	82.1	89.1	2.0	1.3	3.3	7.3	0.4	7.7	546
River fish	22.9	63.9	86.8	0.4		0.4	12.8		12.8	266

Previously Spawned Fish

The salmon which had formerly spawned on one occasion and were again entering the river to spawn for the second time have been separated according to their sea age when entering the river for the first time (table IV). Each sea group is then divided into those fish which have remained away from the river less than a year (short absence fish) and those fish which have been absent for more than a year (long absence fish) since first spawning. In the short absence fish no winter band had formed on the scale since the spawning mark was produced by the first spawning migration. In the long absence fish at least one winter band is present on the scale since the formation of the spawning mark.

It might be pointed out that we did not find a winter band immediately following a spawning mark on any of the scales of Miramichi fish. This would mean that kelts (term applied to the salmon immediately after spawning) seldom return to the sea in the same fall of their spawning migration, but must remain in the river over the winter and return to the sea the following spring. Or, if they return to the sea in the same fall of their spawning, they do not feed until the following summer. This is in agreement with the fishermen's story which is to the effect that they never see any kelts leaving the river in the fall. They claim, however, that they can be seen under the ice in the winter.

Of the 164 salmon in the 1 S.M. group, 68.3 per cent have first spawned as grilse, 29.9 per cent spawned for the first time after their second winter at sea, and there were only three (1.8 per cent) which spawned after their third winter in the sea. Therefore, it is very evident that most of the 1 S.M. group came originally from the grilse.

TABLE IV. Numbers of combined drift and river fish with one spawning mark showing different life histories previous to and following first spawning

First spawning	1st sea winter (grilse)			2nd sea winter (salmon)			3rd sea winter	
Absence after spawning	Short	Long	Total	Short	Long	Total	Short	Total
June 1–15....		8	8					8
16–30....		22	22		4	4		26
July 1–15....		7	7	1	4	5		12
16–31....		21	21		6	6	1	28
Aug. 1–15....		24	24	3	6	9	1	34
16–31....	1	9	10	5	8	13		23
Sept. 1–15....		11	11	1	5	6	1	18
16–30....		8	8	3	1	4		12
Oct. 1–15....		1	1	1	1	2		3
Total.....	1	111	112	14	35	49	3	164

In regard to short and long absence after first spawning, there were 11 per cent with the former behaviour and 89 per cent with the latter. This agrees with the 85 per cent of long absence fish for the Miramichi river as calculated by Huntsman (1931) from the returns of tagged salmon. Of the 89 per cent of long absence fish there were none which stayed away two or more winters.

Of the fish which first spawned as grilse only one, or 0.9 per cent, had a short absence. Of those which spawned after their second winter in the sea,

28.6 per cent were short absence fish. All of the fish spawning after their third winter at sea were short absence fish. Therefore, the older a fish is the more likely is it to return after a short absence in the sea. This condition is the same as that found by Huntsman (1931) for the Margaree fish. He says:—"There is a definite indication that the larger fish are more apt to spawn in a year's time than the smaller fish." It seems that the grilse or younger sea groups require a greater time to recover from the effects of spawning than the older sea groups do. Also it is quite noticeable from table IV that the short absence fish return to the river later in the year than do the long absence fish. Only two of the 18 short absence fish returned before the end of July.

In table V are shown the fish with two spawning marks and which, therefore, are returning on their third spawning migration. These are grouped as above according to their sea age at first spawning and the length of time between successive spawnings. For example, short-long refers to a short absence (less than a year) after the first spawning, and a long absence (more than a year) after the second spawning.

TABLE V. Numbers of combined drift and river fish with two spawning marks showing different life histories previous to and following first and second spawning

First spawning	2nd sea winter				3rd sea winter	Total
Absence after spawning	Short-short	Short-long	Long-short	Total	Long-short	
June 1–15	1			1		1
16–30						
July 1–15		1		1		1
16–31						
Aug. 1–15	4		1	5		5
16–31	2		1	3	1	4
Sept. 1–15	1			1		1
16–30	1			1		1
Oct. 1–15						
Total	9	1	2	12	1	13

There were only 13 fish with two spawning marks, which is 0.9 per cent of the entire catch. Twelve of these spawned for the first time after the second sea winter and the remaining one spawned for the first time after the third sea winter. It seems strange that none of these fish spawned for the first time as grilse since this group formed the greater proportion (68.3 per cent) of the fish with one

spawning mark. So it appears that the fish which mature at an early age (such as the grilse) do not have as good a chance of surviving to a ripe old age as do the fish which mature at a later age.

The fish with two spawning marks also differ from the fish with one spawning mark in the proportion of short absence to long absence fish. In the 2 S.M. group we find that 12 out of 13, or 92.3 per cent, had a short absence between their second and third spawning migrations, while only 11 per cent of the 1 S.M. group had a short absence between their first and second spawning migrations. However, it is in agreement with the statement made previously that the older a fish is the more likely is it to return after a short absence in the sea. Again, we might point out that only one of the 12 short absence fish returned before the end of July. This fish was a mending kelt taken in the drift nets on June 13; it was a female with the caudal fin greatly worn from the spawning operations of the previous fall. The scale showed only one or two ridges of growth for the current summer.

Most of the fish with two spawning marks have the same length of absence on both occasions, as 9 out of 13 are classified as short-short. There were three with a long-short absence, while only one, which was taken in the drift nets, was in the short-long category.

SMOLT AGES (RIVER AGES)

To be consistent with the term employed to designate the sea life, viz., sea ages, we should refer to the river life as river ages, but the term smolt age is more commonly used. When the parr are ready to leave fresh water they discard their parr dress of dark bands to become in appearance like the larger salmon in miniature. They are then called smolts. So smolt ages merely refer to the age of the young salmon when leaving the fresh water.

In table VI will be found the proportion of smolt ages in the total of all sea groups for succeeding half-monthly periods throughout the season of 1931. In the total of 1,557 fish for all the sea groups, the three-year smolts are very predominant with a percentage of 78.1. The two-year smolts come second in importance, forming 15.1 per cent, while the four-year smolts make up only 6.6 per cent of the total. Only three (0.2 per cent) five-year smolts were found; two of these were taken in the drift nets in June while the other was from the river in October. These proportions of smolt ages are practically the same as for the Restigouche river (Phelps and Belding 1930). In the Cascapedia river the smolts are considerably older, since 34.1 per cent were four-year smolts (Calderwood 1927). In the Saint John and Minas systems we have the younger smolts predominating (Huntsman 1931). In the Moisie river the three-year smolts only slightly predominate over the two-year smolts (Macfarlane 1928).

The proportion of two-year smolts in the total increases somewhat with the advance of the season, although the high percentage of 16.8 in June does not correspond with this increase. From 8.7 per cent in the latter part of June they increase to 19.4 per cent in October. On the other hand the four-year smolts

TABLE VI. Percentage of smolt ages in total of all sea groups during successive half-monthly periods for combined drift and river fish

Smolt age	2	3	4	5	Total no.
June 1–15	16.8	77.4	5.8		137
16–30	8.7	80.6	10.0	.6	310
July 1–15	15.1	79.0	5.9		205
16–31	14.0	77.8	8.2		257
Aug. 1–15	16.5	77.2	6.3		224
16–31	18.9	74.1	7.0		143
Sept. 1–15	16.3	81.4	2.3		129
16–30	22.4	74.1	3.4		116
Oct. 1–15	19.4	77.8		2.8	36
Total	15.1	78.1	6.6	.2	1557

show a slight decrease with the advance of the season. Does this mean that the younger fish (two-year smolts) require a slightly longer sojourn in the sea before reaching maturity than the older fish (four-year smolts) do? If so, the percentage of two-year smolts in each succeeding and older age group should increase while the percentage of four-year smolts should decrease. The percentage of smolt ages in each sea group will be found in table VII. The two-year smolts do increase from 13.2 per cent in the 1+ sea group to 16.3 per cent in the

TABLE VII. Percentage of smolt ages in each sea group for combined drift and river fish

Sea group	Smolt age				Total no.
	2	3	4	5	
1+	13.2	79.3	7.5		174
2	16.3	66.7	17.0		135
2+	14.4	80.2	5.2	.2	1042
3	33.3	57.1	9.5		21
3+	37.5	62.5			8

2 sea group, but to only 14.4 per cent in the 2+ sea group. The percentages in the 3 and 3+ sea groups are 33.3 and 37.5 respectively. The four-year smolts decrease from 7.5 per cent in the 1+ sea group to 5.2 per cent in the 2+ sea group, but in the 2 sea group they are much more numerous than in either of these groups, forming 17 per cent. So we might say that the figures regarding

the tendency for the younger smolts to return to the river later than the older smolts are not very conclusive. This phenomenon commonly occurs in the Atlantic salmon of Scotland. Calderwood (1927) found it did not apply to the Cascapedia salmon. A slight tendency was noted by Menzies (1925*b*) in the Moisie salmon.

SUMMARY

In a sample of 1,557 fish for the entire drift and river fishery in 1931, the groups based upon years spent in the sea were 1+ (grilse), 2 and 2+, and 3 and 3+ sea groups; and those based upon previous spawning were 1 S.M. and 2 S.M. groups.

The grilse did not enter the river until June, with the maximum run for the season (until the end of August) during the latter part of June. The ordinary salmon entered the river early in May and reached a maximum around the middle of June.

Excluding the grilse the 2 and 2+ sea groups are those most important (together forming 85.1 per cent of the total), then the previously spawned fish (12.8 per cent), and lastly the 3 and 3+ sea groups (only 2.1 per cent).

The 2 group is comparatively more abundant in June and decreases gradually during the remainder of the season. The 2+ group is the most numerous one for all parts of the fishery season. During the two months of June and July the 2 group is much better represented in the river fishery than in the drift net fishery and vice versa for the 2+ group, which fact shows a definite movement of the 2 group from the drifting area into and up the river.

The proportion of the 3 group in the total is much greater at the beginning and at the end of the season than during the summer months.

The proportion of salmon in the 1 S.M. group increases more or less with the advance of the season. The low percentage of this group at the beginning of the season is partly due to the influence of the drift fishery, in which it forms 7.3 per cent, as compared with 12.8 per cent in the river fishery.

Of the 1 S.M. group, there were 68.3, 29.9 and 1.8 per cent first spawning as 1+, 2(+) and 3(+) respectively; only 11 per cent were returning in less than a year after first spawning (short absence). The older the fish the greater was the proportion returning after short absence, there being 0.9, 28.6 and 100 per cent among those 1+, 2(+) and 3(+) respectively at first spawning. Moreover in the 2 S.M. group 92.3 per cent had a short absence before the last return. That those first spawning as grilse formed the greater proportion (68.3 per cent) of the 1 S.M. group but were absent from the 2 S.M. group indicates that fish maturing at an early age do not live so long as those maturing later. Most of the 2 S.M. group had the same length of absence on both occasions, 9 out of 13 being classified as short-short.

Of the four smolt ages among all fish, the three-year smolts were predominant (78.1 per cent) and followed in order by two-year smolts (15.1 per cent), four-year smolts (6.6 per cent) and five-year smolts (0.2 per cent).

REFERENCES

CALDERWOOD, W. L. *Proc. Roy. Soc. Edin.* **47**, 142-147. 1927.

HUNTSMAN, A. G. *Biol. Bd. Can. Bull.* **21**. 1931.

MACFARLANE, P. R. C. *Proc. Roy. Soc. Edin.* **48**, 134-139. 1928.

MENZIES, W. J. M. The salmon: its life story. Wm. Blackwood and Sons, Edinburgh and London. 1925*a*.

Proc. Roy. Soc. Edin. **45**, 334. 1925*b*.

MENZIES, W. J. M. AND P. R. C. MACFARLANE. *Proc. Roy. Soc. Edin.* **47**, 359-365. 1927.

PHELPS, E. B. AND D. L. BELDING. An investigation of certain conditions affecting the salmon fisheries of the bay of Chaleur and adjacent waters. The Evening Post Job Printing Office, Inc., New York, N.Y. 1930.

Tidal Mixing in an Estuary

By H. B. Hachey
Atlantic Biological Station

(*Received for publication August 7, 1934*)

ABSTRACT

Large scale mixing in the region of the tidal reversing falls of the Saint John river water with salt bay of Fundy water gives light mixed water traceable during spring freshets as far as Grand Manan. This water when moving out may cause renewal of the bay of Fundy waters, at a rate of over 4,000,000 cu. ft./sec. (over 97,000,000 litres/sec.).

INTRODUCTION

Tidal mixing in estuaries is an important factor in determining the character of inshore waters, and, while invariably occurring, is extremely variable in degree. The importance of the mixing will depend upon: (*a*) its extent, determined principally by tidal amplitude and the configuration of the estuary; and (*b*) the amount of drainage water from the river system.

The estuary of the Saint John river in southern New Brunswick offers a striking and in some respects a simple case to demonstrate the mechanism that may be involved, and the effect of the mixing on the adjacent waters.

THE ESTUARY OF THE SAINT JOHN RIVER

The Saint John river, which empties into the bay of Fundy from the north, has a drainage area of approximately 21,500 sq. mi. (55,040 sq. km.) (Canada Year Book, 1926). The discharge of fresh water from the river to the bay of Fundy may be calculated from published (1930) and unpublished data of the Department of the Interior of Canada. Current measurements at Pokiok, on the Saint John river, have been made over a period of years by the Department of the Interior. The drainage area of the Saint John river above Pokiok is given (1930) as 15,300 sq. miles. To obtain the total discharge of the Saint John river the figures for Pokiok were multiplied by 1.4, which factor represents the ratio of the total drainage area of the Saint John river, to the drainage area of the Saint John river above Pokiok. The mean discharge of the Saint John river into the bay of Fundy, as calculated for the period 1918-32, is approximately 33,000 cu. ft/sec. (729,000 l./sec.). At the time of spring freshets (April and May), the mean discharge, as calculated, amounts to 66,800 cu. ft./sec. (1,670,000 l./sec.).

The rise of the tides in Saint John harbour is 26 ft. (8.1 m.) at springs, and 22.8 ft. (6.9 m.) at neaps (Tide Tables 1933). At Fredericton, a distance of approximately 70 mi. (112 km.) up the river, the tidal rise may be as much as 7 in. (18 cm.).

In its lower course, the Saint John river cuts a deep narrow gorge through a belt of hard rock before it enters the sea. In this gorge, the great rise and fall of the tides of the bay of Fundy produce what is known as the "reversing falls" (see figure 1). This gorge, situated in the estuary (tidal mouth of the river), connects the lower basin (Saint John harbour) with the upper basin (the river proper). At half tide the ordinary current of the river flows out through this narrow gorge in the form of a rapid, but when the water in the lower basin falls 10 or 12 ft. (33 or 40 m.) lower, the rapid becomes a foaming waterfall. This continues until the tide turns and the water in the lower basin rises again to the river level when the rapid disappears. As the tide rises, the level of the water in the lower basin may be 10 or 12 ft. (33 or 40 m.) above that of the upper basin, and a strong current then sets inward through the gorge, which becomes a rapid or even a waterfall when the tide is at its height. The high tides, the configuration of the gorge and its approaches, and the relation between the level of the water in the river proper and mean tide level, in Saint John harbour, bring about this reversal of the direction of the current in the gorge, producing the phenomenon of the "reversing falls". The outward run of water begins approximately 2½ hours to low water, and lasts until approximately 4 hours after low water (Tide Tables 1933). Slack water lasts approximately 1 hour. The inward run begins at approximately 1 hour to high water, and lasts until approximately 2½ hours after high water. The region of the "reversing falls" in the tidal mouth of the Saint John river is thus one where river and tidal waters are brought together, under conditions which make for mixing of considerable quantities of these waters.

THE MIXING MECHANISM

In order to investigate the process involved in the mixing mechanism of the "reversing falls", a series of stations in the tidal mouth of the Saint John river were occupied over a rising tide and a falling tide respectively. The locations of the stations are shown in figure 1. Observations were made on June 12 and June 13, 1930 but, for purposes of presentation, the observations (see table I) will be dealt with in the inverse order.

On June 13, 1930, high water was predicted for 1.26 p.m., and low water for 7.40 p.m. (Tide Tables 1930). Station 7 was occupied at 1.37 p.m. The various stations were occupied in inverse numerical order, and the series was completed with the occupation of station 1 at 5.33 p.m. The distribution of temperature and salinity is shown in section in figures 2(*a*) and 2(*b*).

On June 12, 1930, low water was predicted for 6.42 a.m., and high water for 12.46 p.m. (Tide Tables 1930). Station 1 was occupied at 7.27 a.m. The various stations were occupied in numerical order and the series was completed

TABLE I. Observations of temperature and salinity in the estuary of the Saint John river

	June 12, 1930				June 13, 1930			
Sta.	Time (a.m.)	Depth (m.)	Salinity (‰)	Temp. (° C.)	Time (p.m.)	Depth (m.)	Salinity (‰)	Temp. (° C.)
1	7.38	0	17.90	10.50	5.43	0	11.46	13.50
	7.38	5	30.55	6.43	5.43	5	31.02	6.52
	7.33	10	31.27	6.23	5.38	10	31.17	6.40
	7.27	20	31.31	6.22	5.33	20	31.24	6.33
2	8.15	0	8.98	13.20	4.54	0	12.25	13.20
	8.15	5	31.09	6.22	4.54	5	31.04	6.41
	8.10	10	31.17	6.21	4.49	10	31.13	6.36
3	8.51	0	5.01	14.40	4.33	0	20.95	12.70
	8.51	5	12.11	12.10	4.33	5	29.36	6.89
	8.46	10	15.88	10.84	4.26	10	30.35	6.57
	8.41	15	17.29	10.33	4.21	15	30.73	6.42
4	9.21	0	4.34	14.50	4.12	0	8.06	14.80
	9.21	5	4.34	14.70	4.12	5	28.69	7.06
	9.16	10	4.33	14.70	4.07	10	29.79	6.76
	9.11	15	4.42	14.60	4.02	20	30.25	6.60
5	10.15	0	2.97	14.90	3.55	0	13.60	12.90
	10.15	5	2.94	14.90	3.55	5	18.10	10.78
	10.10	10	2.94	14.90	3.50	10	28.49	6.81
	10.05	25	2.88	14.90	3.44	20	29.97	6.69
6	11.22	0	1.46	15.60	2.51	0	3.64	16.00
	11.22	5	1.46	15.44	2.51	5	8.53	13.70
	11.16	10	1.73	15.20	2.46	10	15.30	11.49
	11.10	25	18.71	9.67	2.40	25	19.27	10.28
	11.04	30	21.42	9.16	2.35	30	19.90	10.05
7	11.54	0	1.10	16.60	1.42	0	1.02	17.70
	11.54	5	0.88	15.49	1.42	5	1.02	16.89
	11.49	10	1.42	14.79	1.37	8	1.21	15.64
	11.42	15	2.36	14.69				

with the occupation of station 7 at 11.54 a.m. The distribution of temperature and salinity is shown in section in figures 2(*c*) and 2(*d*).

It may be pointed out that the two series of observations for the Saint John estuary, as outlined above, do not represent corresponding stages of ebb and flood, station 7 being very nearly at high water in both series.

In figures 2(*a*) and 2(*b*), waters of a temperature of 6.33° C. to 7.00° C., and of a salinity of 30.00‰ to 31.24‰ are found extending to the region of intense mixing (in the neighbourhood of the "reversing falls") at depths below 5 metres. A small portion of the section is occupied by water of a salinity less

than $3.00^0/_{00}$ and of a temperature greater than 14.00° C. The greater part of the section consists of mixed water of a temperature between 7.00° C. and 14.80° C., and of a salinity between $3.00^0/_{00}$ and $30.00^0/_{00}$.

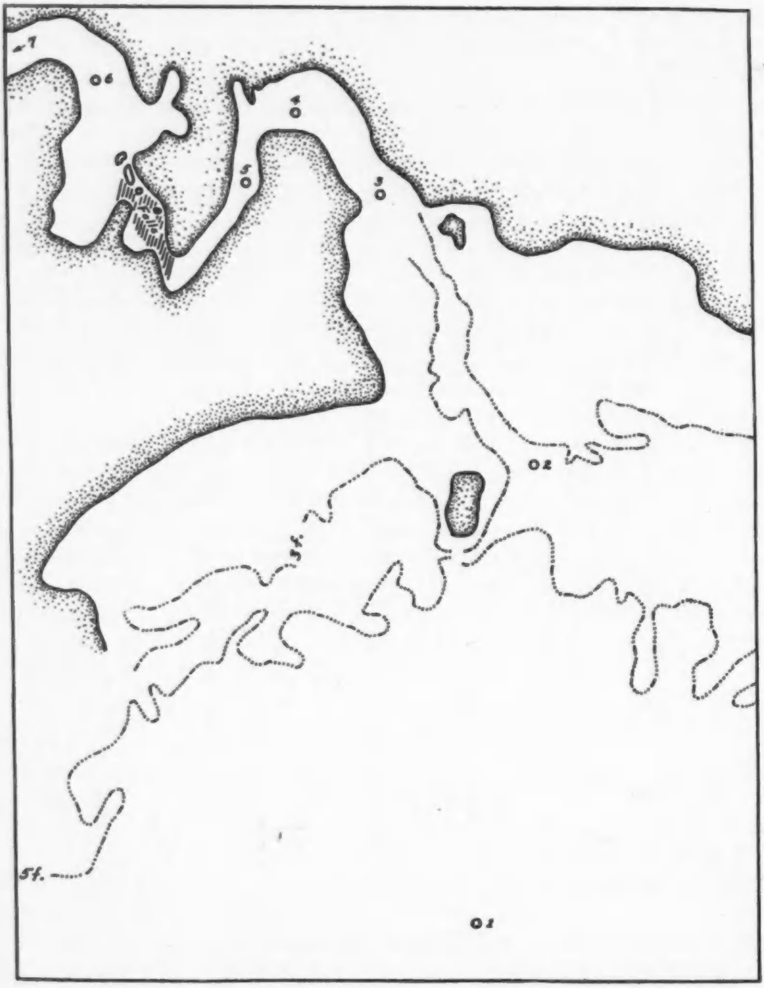


FIGURE 1. Location of stations in the tidal mouth of the Saint John river.

In figures 2(c) and 2(d), it is seen that the colder waters have receded from the area of intense mixing (in the neighbourhood of the "reversing falls") and occupy only a small portion of the section. Water of a salinity less than $3.00^0/_{00}$ and of a temperature greater than 14.00° C. has entered the section from the

river proper. Mixed water is found trapped at the greater depths above the falls, and also occupying an intermediate position between warm fresh waters and cold salt waters.

In the mixing, which takes place in the region of the reversing falls, the efficiency of the mechanism will depend upon a ready supply of the waters to be mixed, and the dispersal of this mixed water from the area of mixing. The observations taken over a rising and falling tide well illustrate the efficient manner in which the mixing mechanism is supplied with the waters to be mixed. Large volumes of heavy salt waters from the bay of Fundy and light fresh waters from the Saint John river are carried to the region of the "reversing falls"

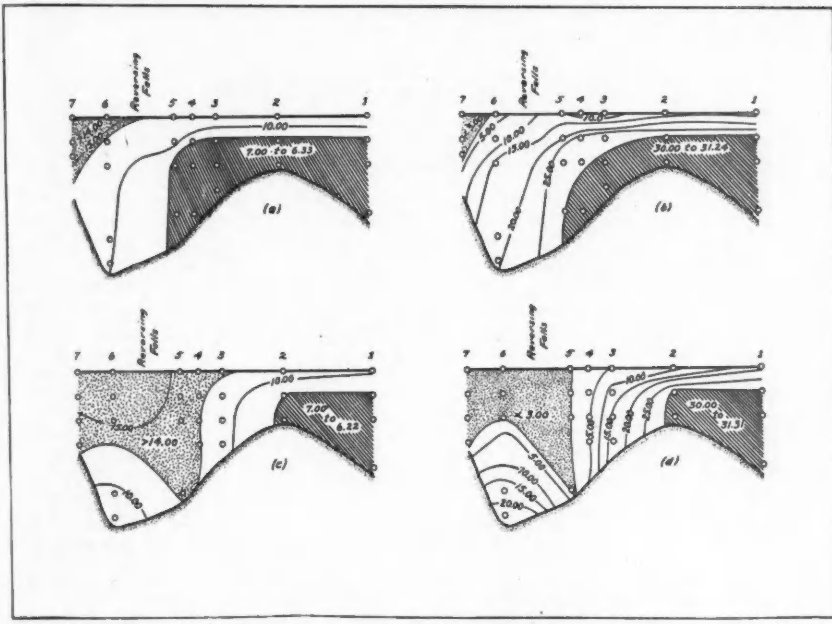


FIGURE 2. Distribution of temperatures and salinities in section: (*a*) temperatures, and (*b*) salinities, June 13, 1930; (*c*) temperatures, and (*d*) salinities, June 12, 1930. Observations at 0, 5, 10, 25 metres, etc.

where mixing takes place. The direction of the surface current in Saint John harbour is nearly always outwards (Tide Tables 1933). As the upper 5 metres of the waters found in Saint John harbour (figure 2) are the result of the mixing (as shown by the salinity), a dispersal of the mixed water from the region of the "reversing falls" is steadily taking place. At a depth of 20 ft. (6.1 m.), the flood stream in Saint John harbour begins 3 hours after low water, and the ebb stream begins 2½ hours after high water (Tide Tables 1933). Consequently the heavy salt waters (figure 2) are carried to the region of the "reversing falls" (at depths

below 5 metres) in the period between 3 hours after low water and 2½ hours after high water.

EFFECT OF SUCH MIXING ON ADJOINING WATERS

The effect of tidal mixing in an estuary, on an adjoining body of water, is well illustrated on a large scale by a consideration of the distribution of the mixed water from the estuary of the Saint John river. The distribution of this mixed water is indicated in figure 3 by means of surface isohalines. The location of these isohalines have been determined from water samples collected between April 17 and May 8, 1930. The pertinent data are furnished in table II. At this time of the year the outflowing water of low salinity is easily traced as far as Grand Manan, a distance of approximately 50 mi. (80.5 km.) from the mixing area of the reversing falls.

TABLE II. Surface salinities for the bay of Fundy

Sta.	Date (1930)	Time	Salinity (°/oo)
873	Apr. 28	1.58 p.m.	32.12
880	Apr. 29	8.17 a.m.	32.05
881	Apr. 29	8.48 a.m.	31.96
956	Apr. 29	2.12 p.m.	32.05
966	......	9.30 a.m.	31.35
967	Apr. 26	10.53 a.m.	31.76
968	Apr. 26	12.03 p.m.	31.92
969	Apr. 17	8.14 a.m.	.....
970	Apr. 17	9.28 a.m.	31.76
971	Apr. 17	10.41 a.m.	31.85
975	Apr. 17	8.40 a.m.	31.53
976	Apr. 17	9.55 a.m.	31.80
977	Apr. 25	11.30 a.m.	31.36
978	Apr. 25	10.55 a.m.	30.28
979	Apr. 25	10.27 a.m.	27.47
980	Apr. 25	9.40 a.m.	30.62
981	Apr. 25	9.12 a.m.	30.30
982	Apr. 26	11.17 a.m.	31.83
983	Apr. 26	9.57 a.m.	31.73
984	Apr. 29	9.47 a.m.	32.00
985	Apr. 29	10.28 a.m.	32.92
986	Apr. 29	11.18 a.m.	31.83
987	Apr. 29	12.23 p.m.	31.73
988	Apr. 29	1.03 p.m.	31.78
989	Apr. 29	1.44 p.m.	31.83
990	May 8	3.04 p.m.	31.04
991	May 8	2.06 p.m.	31.60
992	May 8	12.59 p.m.	32.61
993	May 8	11.54 a.m.	32.61

The high tides of the bay of Fundy result in large and complicated systems of currents. The resultant movement or circulation, however, is effective in controlling the character of the waters in a particular locality. Water is carried

into, and out of the bay of Fundy, during each tidal cycle. Due to the inflow of fresh water from the many drainage areas, it follows that the outflow of water from the bay of Fundy is greater than the inflow (evaporation is not considered of sufficient magnitude to compensate for the surplus from the various drainage areas). The waters of the outflow are made up in part by the waters proceeding from the estuary of the Saint John river. As seen in figure 3, this outflow keeps to the surface and can be traced for a distance of about 50 mi. (80.5 km.). The mixing that takes place in the estuary of the Saint John river consumes considerable quantities of bay of Fundy waters, giving water that eventually proceeds from the mixing area at the surface. This drain on the deep

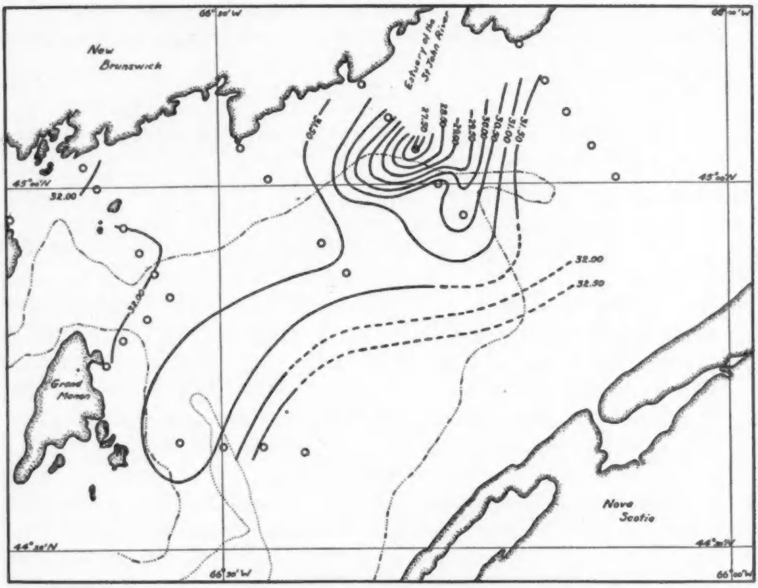


FIGURE 3. Surface isohalines, bay of Fundy, April-May, 1930.

and salter part of the water of the bay of Fundy must be made good by constant renewal from without the bay, and the extent of this renewal will depend upon the amount of the salter bay of Fundy water that the reversing falls consumes.

In spring freshets this consumption is very great, and consequently the renewal of the bay of Fundy waters from without must be on a large scale as is well illustrated by the following figures.

The surface water enclosed by the isohaline of $31.50^{0}/_{00}$ (figure 3) is fairly definitely connected with the outflow from the estuary of the Saint John river. Hence the outflowing water from the bay of Fundy is of a salinity of at least as high as $31.50^{0}/_{00}$.

The surface water enclosed by the isohaline of 32.00‰ and the coast of Nova Scotia (figure 3) is of a salinity greater than 32.00‰. Hence the inflowing water to the bay of Fundy is of a salinity of at least as low as 32.00‰.

The average outflow from the Saint John river to the bay of Fundy (data from sources mentioned previously) for the months of April and May is 66,800 cu. ft./sec. (1,580,800 l./sec.).

The rate of inflow to the bay of Fundy from the open sea for the months of April and May (the amount required to mix with Saint John river waters only in order to produce the outflowing current of a salinity of 31.50‰) is 4,108,200 cu. ft./sec. (97,188,000 l./sec.).

Such an inflow can be represented by a stream 20 mi. (32.2 km.) in width, 20 fm. (36.4 m.) in depth, and with a velocity of 5 mi. (8.1 km.) per day. Such a rate corresponds with the known circulation from drift bottle investigations in the bay of Fundy.

SUMMARY

1. The mixing mechanism in the estuary of the Saint John river operates as follows:

(*a*) The high tides of the bay of Fundy, the configuration of the gorge in the tidal mouth of the river, and the relation between the level of the water in the river proper and mean tide level, bring about a reversal of the direction of the fall of water during a tidal cycle. These factors combine to furnish an area where large quantities of light fresh water are mixed with large quantities of heavy salt water from the bay of Fundy; (*b*) waters from the bay of Fundy are brought to the mixing area (in the region of the reversing falls) at depths below five metres; (*c*) the light mixed water is carried away from the mixing area (in the region of the reversing falls) in the upper five metres.

2. The mixed water proceeding from the mixing area can be traced as far as Grand Manan at times of spring freshets.

3. At times of spring freshets the renewal of bay of Fundy waters may take place at the rate of over 4,000,000 cu. ft./sec. (over 97,000,000 l./sec.). This is equivalent to a stream 20 mi. (32.2 km.) in width, 20 fm. (36.4 m.) in depth, and with a velocity of 5 mi. (8.1 km.) per day.

REFERENCES

CANADA, DEPT. OF THE INTERIOR. *Water Res. Paper* **63**, Ottawa, 91 pp. 1930.

CANADA, DEPT. OF MARINE. *Tide Tables for St. John, N.B.*, **1930**, Ottawa. 1930.
1933, Ottawa. 1933.

Canada Year Book, Ottawa, 1057 pp. 1926.

The Non-Protein Nitrogenous Constituents of Fish and Lobster Muscle

By James Campbell

Department of Physiology, McGill University

(*Received for publication October 26, 1934*)

ABSTRACT

Cod, haddock, salmon, herring, skate and lobster, were investigated, the first two alone proving to be closely related chemically. In the first four the total non-protein nitrogen is of the same order; but in skate it is 3½ times as great, due to urea, ammonia and other volatile bases, and in lobster 1½ times as great, due to the monoamino acid and histidine-arginine fractions. Cod is very rich in the lysine fraction and poor in the histidine-arginine fraction, the reverse of the condition in ox. In herring this difference is also present but is not so great. In lobster the lysine fraction is slightly less than in cod but the nitrogen of the histidine-arginine fraction is extremely high.

The chemical composition of fish muscle especially with regard to the nitrogenous constituents has been less studied than that of the muscles of warm-blooded animals. It is obvious that such knowledge is important in comparative zoology and physiology. The significance of such studies in dietetics is also great as the character of the non-protein nitrogenous constituents of muscle determines to a large extent its digestibility and dietetic value and possibly its secretagogue effect.

For a review of the literature on the composition of fish muscle the paper of Kapeller-Adler and Krael (1930) may be consulted. The composition of haddock muscle has been investigated by Komarov (1933).

The muscles chosen for study were taken from cod (*Gadus callarias*), haddock (*Melanogrammus aeglefinus*), salmon (*Salmo salar*), herring (*Clupea harengus*) skate (*Raja stabuliforis*) and lobster (*Homarus americanus*). These are all examples of rather different types of muscles except that of cod and haddock, which are of the same family. All are important commercially in the Canadian fisheries, with the exception possibly of skate muscle. Lobster muscle, representative of crustaceans, has a most powerful secretagogue effect on the gastric glands, as has been found by Alley (1933), and this indicated a possible variation in the nature of the non-protein nitrogen from it.

The extracts of cod, herring and lobster muscle were more particularly studied by the silver-baryta fractionation procedure of Kossel and Kutscher

devised for the isolation of the nitrogenous bases (see Hoppe-Seyler Thierfelder 1924).

METHODS

Crude Extracts

The tissue could not always be obtained from perfectly fresh fish. This was possible in the case of skate and lobster muscle which was taken immediately after killing the animals. In the case of lobster particularly, all possible speed was made so that in 30 minutes after death the muscle had been rapidly heated to 80° C. The "wing" muscle of the skate and from the lobster the muscle of the cephalo-thorax as well as that of the claws were used. The muscle fillets of the other fishes were dissected out and extracted immediately after they were received, but previous to this the cod and haddock had been dead about three hours, the herring had been kept in the holds of small fishing vessels for about 18 hours after capture, and the salmon had been dead from two to four hours.

The muscle fillets were removed without contamination from skin, scales or slime and then minced. To approximately 600 g. portions in 2000 cc. beakers were added 2 volumes of distilled water and 0.5N HCl to bring to pH 5.0. Under the conditions of the experiment maximum precipitation of protein occurred at this point. The beakers, placed in vigorously boiling water baths and constantly stirred, reached a temperature of 80° C. in about 15 minutes and this extraction was continued for 20 minutes. The material filtered hot through several layers of gauze on a Buchner funnel gave a clear yellowish filtrate. The cake on the funnel was pressed and washed with hot water, then it was extracted in the same way another time with $1\frac{1}{2}$ volumes of distilled water at pH 5.0, filtered, washed and extracted once more with 1 volume of water. The extracts were evaporated separately "in vacuo" in the presence of thymol at a temperature of the water bath not exceeding 60° C. After evaporation to such a volume that 1 cc. corresponded to 1.5 g. tissue, the material was filtered, the precipitates well washed and the combined extracts evaporated again. In cases where the extract had to be kept for some time an equal volume of 95 per cent ethyl alcohol was added.

Salmon muscle presented difficulties in this treatment since a fine suspension formed which was extremely difficult to separate either by filtration or centrifuging. Treatment with alcohol, acetone or light petroleum failed to improve the separation. If the tissue was first heated for 30 minutes in beakers in the boiling water bath a surprising result was obtained, as, on treatment as described above, filtration became rapid and the filtrates were clear. This was the method finally adopted for the extraction of salmon muscle.

The salmon and herring extracts were subsequently extracted with toluol and ether in a separatory funnel to remove fatty materials. These toluol and ether extracts were themselves shaken with water and the aqueous layer returned to the main extract in each case, so that no loss of water soluble material occurred.

Analysis of Crude Extracts

In the following determinations the extracts were diluted to contain approximately 1 g. tissue per 1 cc.

Determinations of the *total non-protein nitrogen* of the extract were made by the macro-Kjeldahl method. The extracts gave a slight precipitate presumably of protein with trichloracetic acid.

The quantity of this protein material was determined by precipitation in a concentration of 5 per cent trichloracetic acid as the difference in the nitrogen contents of the original solution and the filtrate. In the crude extracts this usually amounted to less than 1 per cent of the nitrogen, and was consequently subtracted from the total nitrogen content of the extract to give the total non-protein nitrogen.

The *volatile base nitrogen* was determined in the apparatus of Van Slyke and Cullen (1916). 2 cc. extract with 10 cc. saturated sodium carbonate were aerated into 5 cc. N/50 HCl which was titrated with N/50 NaOH. From this was subtracted the nitrogen due to ammonia.

The *ammonia nitrogen* was determined by aeration of an aliquot of extract as in the volatile base determination followed by nesslerisation.

Urea nitrogen was determined by the urease method, the ammonia formed being aerated into N/50 HCl as in the volatile base determination. The increase in the titration figure of the urea nitrogen determination over that of the volatile base represents the ammonia due to urea.

The *amino nitrogen* was determined by formol titration under carefully controlled conditions embodied in the method of Northrop (1926). The ammonia nitrogen was subtracted from the titration value.

Creatinine together with creatine were determined by the procedure of Benedict (1914) and Folin (1914).

Imidazole groups were first determined by the procedure of Koessler and Hanke (1919), but later the modifications of Jorpes (1932) were adopted, the data being calculated as the amount of nitrogen in the imidazole group.

These determinations were carried out on extracts of cod, haddock, salmon, herring, skate and lobster muscle.

Fractionation

Further information was obtained by fractionation of the crude extracts of cod, herring and lobster muscle by the silver-baryta method of Kossel and Kutscher (Hoppe-Seyler Thierfelder 1924) with modifications introduced to obviate certain of the disadvantages of the original method, such as the hydrolysing action of hot saturated barium hydroxide and the oxidising action of silver oxide. The method followed is the same as that used by Komarov (1933). He states that the method ". . . is not strictly exact nor truly quantitative. Nevertheless this method appears to be very reliable, since even in different hands it gives average figures of great uniformity when applied to the same tissue."

In this way the original extract was separated into two main fractions, (1) *nitrogenous bases* (phosphotungstic acid precipitate), and (2) "*monoamino acid fraction*" (phosphotungstic acid filtrate).

The nitrogenous base fraction was further subdivided into three fractions:

(*a*) ***purine fraction*** (first silver precipitate); (*b*) ***histidine-arginine fraction*** (second silver precipitate); (*c*) ***lysine fraction*** (second silver filtrate reprecipitated by phosphotungstic acid).

In the separation of the fractions 1 and 2 there is a loss of nitrogen in the precipitates which is not recovered by careful and repeated washing. This "humin nitrogen" of the 1st phosphotungstic acid precipitate is designated "humin nitrogen 1."

A further loss occurs on further fractionation of the nitrogenous bases. The total loss, including the "humin nitrogen 1" may be designated "total unrecovered nitrogen" or "total humin nitrogen".

As it was found that the purine fraction, particularly of cod muscle, appeared to contain material of proteose and peptone nature, this was determined in a portion of the fraction, in each case, by tannic acid precipitation according to the method of Wasteneys and Borsook (1924). This nitrogen, precipitable by tannic acid, was subtracted from the non-protein, the nitrogenous base and purine fraction nitrogen.

The data are presented in terms of milligrams of nitrogen per 100 g. wet weight of tissue (designated mg. per cent) and as per cent of the total non-protein nitrogen (designated per cent).

RESULTS

The results of these analytical procedures are included in tables I and II. The determinations of table I were made with extracts from different lots of tissues than those of table II, with the exception of lobster muscle, in which the same extract served for the data of both tables.

TABLE I. Non-protein nitrogenous constituents of muscle. Results are expressed as mg. nitrogen per 100 g. wet weight muscle and as per cent of the total non-protein nitrogen.

Nitrogen in the form of	Haddock		Cod		Salmon		Herring		Skate		Lobster	
Non-protein	417.0	100.0	400.0	100.0	466.0	100.0	425.0	100.0	1423.0	100.0	682.8	100.0
Volatile base (other than ammonia)	7.6	1.82	2.8	0.70	0.6	0.13	4.7	1.11	152.1	10.7	0.6	0.09
Ammonia			9.5	2.4	11.9	2.55	9.3	2.19	35.4	2.69	19.9	2.92
Urea	2.0	0.48	2.3	0.60	1.3	0.28	1.7	0.40	(800.0)	(56.0)	0.07	0.010
Amino	28.3	6.79	34.2	8.55	39.1	8.03	45.4	10.7	52.9	3.51	211.0	30.9
Creatine and creatinine	205.0	49.2	169.0	42.2	193	41.4	185	43.5	107.6	7.56	4.67	0.68
Imidazole group	Trace		Trace		2.36	0.51	28.1	6.61				

The main result of the present investigation is that muscle tissue of different species shows remarkable differences in the concentration of total non-protein nitrogen and especially in its nature.

TOTAL NON-PROTEIN NITROGEN

The values of non-protein nitrogen given in table I are probably slightly affected by the formation of proteoses and peptones, through the extraction of the muscle with hot water. Proteoses were found in extracts of meat by Fürth and Schwartz (1911) and are regarded as artificial products formed as a result of

hydrolysis occurring during the extraction of the tissue with hot water. These forms of nitrogen were taken into account only in table II.

The values of non-protein nitrogen for haddock, cod, salmon and herring muscle are of the same order, with perhaps a slightly greater amount present in salmon and herring muscle. The non-protein nitrogen of the muscle of warm-blooded animals is also of this order (values are reported from 330 mg. per cent to 450 mg. per cent). In contrast to this group of similar values the amount of non-protein nitrogen in skate muscle is enormous, being 3.5 times greater. Lobster muscle also has the much higher value of 682.8 mg. per cent or an increase of 50 per cent over the value for the first group.

Distribution of Nitrogen

VOLATILE BASE AND AMMONIA NITROGEN

The ammonia nitrogen and the other volatile base nitrogen do not show any notable changes in haddock, cod or salmon muscle. In herring the volatile base nitrogen other than ammonia is higher than in the former cases, while lobster shows a fairly high content of ammonia nitrogen. Skate muscle is extremely rich in ammonia and other volatile base nitrogen.

These volatile bases are probably chiefly composed of trimethylamine (Kapeller-Adler and Krael 1930).

UREA NITROGEN

In haddock, cod, salmon and herring muscle the urea nitrogen values do not vary greatly (1.3 to 2.3 mg. per cent). In skate the concentration is enormous, but as is indicated by brackets, this figure is known only approximately. Lobster muscle is singularly poor in urea.

AMINO NITROGEN

The amino nitrogen of herring (45.4 mg. per cent) is greater than that of haddock, cod and salmon (28.3 to 39.1 mg. per cent). In skate muscle the value is high (52.9 mg. per cent) and in lobster it is extremely high (211 mg. per cent) being four times that of skate. The percentages of this amino nitrogen are also interesting. In haddock, cod and salmon muscle it forms 6.79 per cent to 8.55 per cent of the total non-protein nitrogen, but in herring 10.7 per cent. In skate the value is only 3.51 per cent due to the large non-protein nitrogen value, but in lobster the value is 30.9 per cent in spite of the amount of non-protein nitrogen.

CREATINE AND CREATININE

Creatine and creatinine seem to be less in skate muscle than in haddock, cod, salmon and herring. The quantity present in lobster muscle is negligible.

IMIDAZOLE GROUP NITROGEN

The imidazole group nitrogen shows extreme variations. It is absent in haddock and cod muscle, present in salmon to the extent of 2.36 mg. per cent and in herring to the extent of 28.1 mg. per cent. This form of nitrogen was un-

fortunately not determined in the case of skate and lobster muscle. The data obtained are in agreement with the findings of Clifford (1921) who also found none of this material in crustacean muscle.

Characteristics of the Muscles

In the group haddock, cod, salmon and herring, of approximately the same amount of non-protein nitrogen in the muscles, cod and haddock are closely alike. In contrast to them salmon contains less urea and some imidazole group nitrogen, and herring more volatile base nitrogen other than ammonia, more amino nitrogen and ten times the imidazole group nitrogen of salmon. The urea nitrogen is almost the same as in salmon.

The characteristics of skate muscle with its high non-protein nitrogen, volatile base and ammonia nitrogen and especially urea nitrogen are well known. The absolute amount of amino nitrogen in skate muscle is also high, but as per cent of the total non-protein nitrogen it is low.

It may be noted that the non-protein nitrogen of skate muscle, less the nitrogen of the volatile bases, ammonia and urea, giving a value of 435 mg. per cent, is not much more than for haddock, cod, salmon and herring which vary from 385 mg. per cent to 452 mg. per cent This figure for lobster muscle however is 662 mg. per cent.

Lobster muscle contains 50 per cent more non-protein nitrogen than the first group. It has little volatile base nitrogen other than ammonia, fairly high ammonia nitrogen and traces only of urea nitrogen. Creatine and creatinine are present only in minute amounts and the amino nitrogen is very high both in absolute amount and as per cent of the total non-protein nitrogen.

Fractionation

The distribution of nitrogen in the muscle extracts of cod, herring and lobster, as studied by the phosphotungstic acid and silver baryta method, was considered to be a particularly important part of the investigation. While the muscles of warm-blooded animals have been extensively investigated by this method (Hoppe-Seyler Thierfelder 1924), no adequate study of various fish muscles has been made. A recent study of haddock muscle by Komarov (1933) brought to light striking differences in the composition of this tissue as compared with the muscle of ox, dog and horse, and it was decided to extend the study to other species of fish.

Under carefully controlled conditions, phosphotungstic acid has a specific property of precipitating quantitatively all the nitrogenous bases and thus provides a means of separating basic nitrogenous compounds from those having acid characteristics. The filtrate after phosphotungstic acid precipitation is referred to arbitrarily as the "monoamino acid fraction".

The precipitate obtained by treatment with phosphotungstic acid and subsequent decomposition with baryta is designated the "nitrogenous base fraction". This fraction is further fractionated by treatment with silver nitrate

and baryta into the purine fraction, the histidine-arginine fraction and the lysine fraction.

A considerable proportion of the nitrogen could not be recovered in the first treatment with phosphotungstic acid and baryta and is designated humine nitrogen 1. This and the further losses in the fractionation of the nitrogenous bases are included in the "total humine nitrogen".

The data obtained by this method (see table II) show profound differences in the composition of cod, herring and lobster muscle.[1]

TABLE II. Distribution of non-protein nitrogen in muscle. (Results are expressed as mg. nitrogen per 100 g. wet weight tissue and as per cent of the total non-protein nitrogen).

Nitrogen in the form of	Cod		Herring		Lobster	
Non-protein	419.2	100.0	437.0	100.0	682.2	100.0
Volatile base (other than ammonia)	2.10	0.50	16.1	3.68	0.60	0.09
Ammonia	7.74	1.85			19.9	2.92
Urea	1.86	0.44	4.00	0.92	0.07	0.010
Amino-	39.2	9.34	48.8	11.2	211	30.9
Creatine and creatinine	163	38.9	182.7	41.8	4.67	0.684
Imidazole group	Trace		19.1	4.37		
Nitrogenous bases	257.6	61.44	269.9	61.77	382.6	56.04
*Purine bases	24.5	5.87	8.21	1.88	10.7	1.57
Histidine-arginine fraction	32.7	7.80	72.7	16.6	191.0	28.0
Lysine fraction	157.9	37.67	145.6	33.32	150.6	22.1
Monoamino acid fraction	95.7	22.8	97.9	22.4	221.5	32.4
Humin nitrogen 1	65.9	15.8	69.2	15.8	78.1	11.6
Total humin nitrogen	108.4	25.9	112.6	25.8	108.4	16.0

*In the form of nitrates.

NITROGENOUS BASES FRACTION

The nitrogenous base nitrogen of herring muscle is greater in absolute amount (269.9 mg. per cent) than that of cod muscle (257 6 mg. per cent), but only a trifle greater as per cent of the total non-protein nitrogen (respectively 61.77 per cent and 61.44 per cent).

Lobster muscle contains a markedly less proportion of the total non-protein nitrogen (56.04 per cent) as nitrogenous bases than in the former cases. Due, however, to the high value of non-protein nitrogen in lobster muscle the absolute amount of nitrogen in this fraction is much greater (387.6 mg. per cent).

The comparison with haddock and ox muscle (Komarov 1933) in this respect is of interest. Haddock muscle contains 239.4 mg. per cent nitrogenous

[1]The analyses previously described (table I) were carried out on a small portion of the extract and the main bulk of it was then fractionated. (Extracts equivalent to 4 kg. cod muscle, 2 kg. herring muscle and 3 kg. lobster muscle were fractionated in this way.)

In the first part of table II, the analyses of cod and herring muscle confirm the values of table I, with the exception of the urea nitrogen of herring muscle which is 4.00 mg. per cent in the former case and 1.7 mg. per cent in the latter. We may again note that herring has a higher value of non-protein nitrogen than cod, apparently more volatile base, higher amino-nitrogen and higher creatine and creatinine value.

base nitrogen or 63.00 per cent of the total non-protein nitrogen, while ox muscle contains 215.4 to 175 mg. per cent or about 48.2 per cent of the total non-protein nitrogen. Haddock muscle is therefore closely like cod and herring muscle but distinct from ox muscle and lobster muscle in this respect.

PURINE BASE FRACTION

The purine bases were in the form of nitrates when the nitrogen determinations were made, and consequently give higher values than would occur if they had been purified into the form of the free bases.

The nitrogen of this fraction is greater in cod muscle than in herring and lobster muscle which are not different in this respect. The amount of nitrogen in this fraction of cod muscle is similar to that found in dog and horse muscle by Fürth and Schwartz (1911).

HISTIDINE-ARGININE FRACTION

The distribution of nitrogen in the histidine-arginine and lysine fractions is so important that it is considered justifiable to make it clearer by the inclusion of table III in which the grouping has been rearranged.

TABLE III

Nitrogen of the	Muscle	Mg. % of wet tissue	% of total non-protein nitrogen
Histidine-arginine fraction	Cod	32.7	7.80
	Herring	72.7	16.6
	Lobster	191.0	28.0
Lysine fraction	Cod	157.9	37.6
	Herring	145.6	33.3
	Lobster	150.6	22.1

These fractions are commonly associated with high physiological activity, the components of the former showing a histamine-like, and of the latter a chlorine-like action.

The nitrogen of the histidine-arginine fraction in cod muscle is very low both in absolute amount and as per cent of the total non-protein nitrogen. Herring muscle contains more than double the amount found in cod muscle, and in lobster muscle it is 6 times as high in amount (mg. per cent) and 3.6 times as high in per cent of the total non-protein nitrogen.

In the investigations of Komarov (1933) the nitrogen of this fraction in ox muscle was found to be 89.8 mg. per cent or 22.3 per cent of the total non-protein nitrogen, and in haddock muscle 51.5 mg. per cent or 13.55 per cent of the total non-protein nitrogen, *i.e.*, about half as much as in the former. In this respect haddock and cod muscle appear to be similar.

The large amount of this form of nitrogen in lobster muscle must be due in part to the presence of arginine which is supposed to take the place of creatine in this muscle (Kutscher 1914).

In ox muscle the bulk of this fraction is composed of carnosine, but although

many attempts to isolate this dipeptide from cod muscle were made, they were entirely without success. This is supported by the negative test for imidazole groups.

Suzuki *et al.* (1909) claim to have found 0.2 per cent of carnosine from the muscle of the Japanese cod or bonito (*Gymnosarda pelamis*).

LYSINE FRACTION

The nitrogen recovered in the lysine fraction of cod muscle is, conversely, very high, both in absolute amount and as per cent of the total non-protein nitrogen. In herring the values are lower both in amount and as per cent of the total non-protein nitrogen; and in lobster muscle, while the amount present is between that of cod and herring muscle, the per cent of the total non-protein nitrogen is much lower.

According to Komarov (1933) the nitrogen of this fraction in ox muscle is 65.6 mg. per cent or 16.3 per cent of the total non-protein nitrogen; and in haddock muscle is 145.0 mg. per cent or 38.15 per cent of the total non-protein nitrogen.

Cod muscle and haddock muscle therefore exhibit a predominance of the lysine fraction over the histidine-arginine fraction in contrast to ox muscle. They are also characteristically distinct from herring and lobster muscle. In this we recognize the family relationship of cod and haddock by the chemical composition of their muscular tissues.

TOTAL HUMIN NITROGEN

During the process of fractionation some nitrogen was lost in spite of all efforts to carry out all the procedures quantitatively, all the precipitates being exhaustively washed out. The behaviour of extracts prepared from cod, herring and lobster muscle was in this respect essentially the same as in the case of extracts of haddock and ox muscle (Komarov 1933).

The total humin nitrogen forms a considerable proportion (25 per cent) of the total non-protein nitrogen in cod and herring muscle, where the values are very close to one another, but a somewhat less proportion in lobster muscle (16 per cent). While in haddock muscle this figure was found to be only 12 per cent (Komarov 1933), the values found in the case of ox muscle by the same investigator are more nearly equal to the results for cod and herring muscle in this work.

MONOAMINO ACID FRACTION

The "monoamino acid fraction" is a rather arbitrary term to designate the material not precipitated by phosphotungstic acid. In herring muscle the amount of nitrogen in this fraction is slightly greater than in cod muscle but the per cent of the total non-protein nitrogen is a trifle less. This is reflected in the inverse variation of the nitrogenous base fraction nitrogen of herring and cod muscle. In lobster muscle the nitrogen of this fraction is strikingly great both in absolute amount and as per cent of the total non-protein nitrogen, as, for

example, it exceeds the amount in herring muscle by 2.3 times. This is in accord with the lower per cent of the nitrogenous base fraction in lobster muscle.

Characteristics of the Muscle

Fractionation of the muscle extracts shows a high proportion of the total non-protein nitrogen in the nitrogenous base fraction of cod and herring muscle, but a notably lower proportion in lobster muscle. Due to the high value of the total non-protein nitrogen of lobster muscle, the absolute amount of this fraction is, however, higher than in the other two cases.

In cod muscle there is a predominance of the lysine fraction over the histidine-arginine fraction. In herring muscle the lysine fraction is also greater than the histidine-arginine fraction, but the difference is not so marked. In an investigation of ox muscle (Komarov 1933) the histidine-arginine fraction was found to exceed the lysine fraction but this condition was found to be reversed in haddock muscle. There is a close correspondence in the partition of nitrogen in haddock and cod muscle.

Lobster muscle is characterised by a very high proportion of nitrogen in the histidine-arginine fraction and in the monoamino acid fraction.

CONCLUSIONS

A chemical investigation of the non-protein nitrogenous constituents of the muscle of cod, haddock, salmon, herring, skate and lobster has been made with more particular attention to cod, herring and lobster muscle in which the partition of nitrogen was investigated by fractionation procedures.

With the exception of cod and haddock muscle, which are closely related chemically, profound differences in the composition of these tissues in many respects have been brought to light.

The chief characteristics of the tissues appear to be as follows. The total non-protein nitrogen of the muscle in the first four cases is of the same order; in lobster muscle there is a 50 per cent increase over this while in skate muscle the amount is three and a half times as great. The constituents responsible for this increase in skate muscle are urea, ammonia and other volatile bases. In lobster muscle the increase is due to the nitrogen of the "monoamino acid" fraction and of the histidine-arginine fraction.

Cod muscle is very rich in the lysine fraction and poor in the histidine-arginine fraction, this being the reverse of the condition in ox muscle. Herring muscle in this respect is intermediate between these two but the lysine fraction is still twice as great as the histidine-arginine fraction. In lobster muscle the lysine fraction is slightly less than in cod muscle but the nitrogen of the histidine-arginine fraction is extremely high.

The position of these forms of fishes in the morphological scheme seems to be related to the chemical composition. As evidence of this it may be noted that cod and haddock, of the same family, have muscles of similar composition, but they are distinctly different, in this respect, from the other forms.

The author is greatly indebted to Dr. B. P. Babkin, under whose direction this work was carried out, and to Dr. S. A. Komarov, who also directed and supervised it. The Halifax, N.S., and the St. Andrews, N.B., Stations of the Biological Board of Canada gave financial and laboratory assistance in the work and to their directors and staffs many thanks are due.

REFERENCES

Alley, A. *Contr. Canad. Biol. Fish.* **8**, 229. 1913.

Benedict, S. R. *J. Biol. Chem.* **18**, 191. 1934.

Clifford, W. M. *Biochem. J.* **15**, 725. 1921.

Folin, O. *J. Biol. Chem.* **17**, 469. 1914.

Fürth, O von and C. Schwartz. *Biochem. Zeit.* **30**, 413. 1911.

Hoppe-Seyler Thierfelder. Physiologisch- u. pathologisch-chemische Analyse. 9e Aufl. J. Springer, Berlin, p. 864. 1924.

Jorpes, E. *Biochem. J.* **26**, 1509. 1932.

Kapeller-Adler and J. Krael. *Biochem. Zeit.* **221**, 437. 1930.

Komarov, S. A. *Contr. Canad. Biol. Fish.* **8**, 125. 1933.

Koessler, K. K. and M. T. Hanke. *J. Biol. Chem.* **39**, 507, 539. 1919.

Kutscher, F. *Zeit. Biol.* **64**, 240. 1914.

Northrop, J. H. *J. Gen. Physiol.* **9**, 767. 1926.

Suzuki, U., K. Joshimura, M. Jamakawa, and Y. Irie. *Zeit. Physiol. Chem.* **62**, 1. 1909.

Van Slyke, D. D. and G. E. Cullen. *J. Biol. Chem.* **24**, 117. 1916.

Wasteneys, H. and H. Borsook. *J. Biol. Chem.* **62**, 1. 1924.

The Growth of the Pacific Edible Crab, *Cancer magister* Dana

By Donald C. G. MacKay and Frank W. Weymouth
Pacific Biological Station, and
School of Biological Sciences, Stanford University, California

(*Received for publication November 27, 1934*)

ABSTRACT

Approximately 20,000 measurements were made of crabs and cast exoskeletons, chiefly from Boundary bay, southern British Columbia.

From size frequencies of crabs under 3 cm. seven modes have been identified as representing the early post-larval instars. Increase in size of animals in the laboratory or in live-wells is significantly less than in nature, and leads to erroneous results when applied to growth.

The increase per moult decreases from about 40 per cent in the early post-larval stages to about 15 per cent in males of 13.5 cm. and 10 per cent in females of 13.0 cm. Above 10 cm. the males increase more per moult. The intervals between moults become progressively longer with increasing size, and tagging experiments indicate that large crabs moult yearly. Probably to reach the maximum size, seventeen and sixteen post-larval instars are required for males and females respectively.

Sexual maturity in female crabs is probably attained during the fourth or fifth year but may occur in the third or the sixth year. The legal size in British Columbia (6½ inches, or 16.5 cm.) is probably attained during the seventh or eighth year. The average duration of life is probably about eight years and the maximum age not more than ten years.

The determination of age and rate of growth in the crab, as indeed with all arthropods, is beset with peculiar difficulties. As is well known, the rigidity of the exoskeleton in the larger crustacea precludes change in the linear dimensions except at the time of ecdysis. As a consequence, the growth "curve" consists of a series of steps, sudden increases alternating with periods in which the dimensions remain unchanged. The same is approximately true of the weight-length relationship since, although profound chemical and histological changes precede and follow ecdysis, the specific gravity varies but little and the volume is fixed between moultings. This complicated method of growth involves unusual physiological mechanisms which are associated with remarkable osmotic changes.

The difficulties inherent in the study of arthropod growth are greatly increased since hard parts corresponding to the scales, bones, otoliths, and shells of other animals are periodically thrown off. In consequence it is impossible to determine age from traces left by seasonal growth as has been done with fish and molluscs.

With the exception of direct records of animals kept in aquaria which, though helpful in a general way, are always open to the criticism that conditions

J. Biol. Bd. Can. 1 (3) 1935.

were more or less unnatural, three types of data are available from which growth may be inferred. Size frequencies may be used in the determination of age during the early part of life. The increase per moult and the intervals between moults found in the males and females of varying sizes may be approximated and the growth curve constructed. Negative evidence of the intervals between moults may be procured from tagging experiments but, since the tags are lost with ecdysis, the growth cannot be directly determined by this method.

The difficulties involved in the construction of a growth curve from these data are evident when one considers that no satisfactory growth curve exists for any crustacean so far studied and many of these animals are of such great economic value that such information is urgently needed.

The phenomenon of arthropod growth has received considerable attention but reference will be made at this time to only three publications. It has been shown by Baumberger and Olmstead (1928) that the volume increase which accompanies ecdysis in the crab takes place rapidly and results from the absorption of water. Six stages were identified in the moult cycle and were called: hard, pillans, about to moult, newly-moulted, soft, and paper shell. For each of these stages, water content and specific gravity were found to be different and a typical condition was found to exist in each. Water content is low in the hard crabs, higher in the pillans, considerably higher in the newly-moulted, and in the later stages shows a marked decrease. A large absorption of water occurs rapidly during moulting and the specific gravity varies inversely to the water content. The osmotic pressure was also investigated and the following equivalent molecular concentrations obtained:

Monterey bay sea water	1.06
Tissue fluids of hard crabs	0.71
Pillans	1.02
Crabs about to moult	1.40
Newly-moulted crabs	1.18

Water absorption is responsible for the change in osmotic pressure from 1.4 molar solution for crabs about to moult to 1.18 molar solution in the newly-moulted crabs.

There is some indication that crabs seek water of lesser density at the time of ecdysis. This may be related to the above facts.

Calvert (1929) summarizes the literature on growth rates in animals and describes his experiments in rearing two species of *Odonata* from egg to adult. It is pointed out that growth in length occurs in insects and certain other arthropods between moults. Consequently, temporary fluctuations in length are to be found in insect studies. Evidence is given as to the variability in number of moults of the same species and it is concluded that the number of instars are not absolute indicators of biological age. The evidence from rearing nine species of dragon-flies indicates a growth rate more rapid than that postulated by various authors. The growth rate in *Odonata* was found to vary irregularly from instar to instar and not to be correlated with food. In the case of the nymphs of *Anax*

junius, as with some other insect larvae, the per cent increase per moult was not found to be greatest in early age. In some of Calvert's records there was a slight decline in the growth per moult although he does not recognize this fact.

In an attempt to reduce the phenomena of arthropod growth to a simple law, Przibram (1930), chiefly on the basis of his studies on the mantid *Sphodromantis* (*Hierodula*) *bioculata* in the Sudan in 1904, claimed that insects doubled their weight at each moult. This doubling of weight was ascribed to a division of all cells in the body, a view for which he adduces no experimental proof and against which there is much evidence. In two bodies of similar shape, one of which has double the mass of the other, the linear dimensions will be as $\sqrt[3]{1}$ to $\sqrt[3]{2}$ or 1.00 to 1.26. Such linear increases (*i.e.* of 26 per cent) he claims to be the rule and when the increase is distinctly greater he maintains that in the evolutionary history one moult has been dropped out.

GROWTH OF SOME OF THE LARGER CRUSTACEA

A review of the information available on the growth of some of the larger crustacea may be considered here.

The American Lobster, Homarus americanus

Although less closely related to *Cancer magister* than the forms later to be discussed, the lobster is nevertheless of considerable interest since it is a large crustacean which, because of its economic importance, has been carefully studied. Herrick (1911) has assembled the available data on the American lobster, *Homarus americanus*, in an extensive and valuable paper. His observations and conclusions on growth are of distinct interest here and will be considered in some detail.

Data bearing on growth have been obtained from young lobsters reared both at Wood's Hole and at Wickford, Rhode Island, from moulting measurements of larger individuals and from estimates on the basis of the intervals between moults in the adult.

From lobsters reared in hatcheries the first 11 to 14 instars have been identified, their sizes measured, and the interval between moults determined. During the first year the lobster moults twelve times with an average increase of 18 per cent per moult and attains a length of 5.3 cm. In this connection Herrick says: "I think it highly probable that lobsters grow more rapidly in nature than when confined in glass jars in the hatchery." Adequate size frequencies of young lobsters, against which these measurements might be checked, do not seem to be available. However, the probability is that those dimensions represent a fairly close approximation to the growth of the first year. Herrick also gives moulting increases for 8 lobsters between 14 cm. and 28.6 cm. in which the average increase is 8 per cent. On these data Hadley, as quoted by Herrick (1911, p. 362), has estimated that male lobsters pass through 36 instars and female lobsters, 30 instars. The male reaches a length of 57 cm. and the female 43 cm. The increase per moult is assumed to fall from 18 per cent to 4 per cent, an estimate for which there is no satisfactory evidence. The "compound interest effect" of the rate of increase through so many moults would be very great. This, together

with the possible error in assuming biennial rather than annual moulting, makes it clear that the entire growth of the lobster is an estimate which may be greatly in error.

The Atlantic Blue Crab, *Callinectes sapidus*

Churchill (1919), in an extensive study of the life-history of the Blue crab, has attempted to determine the growth rate from his own data together with that of other investigations. Hay had previously procured 22 moulting records; and Binford, at Beaufort, North Carolina, had raised one crab from the *megalops* to the 6th post-larval instar. Churchill himself procured 27 moulting records from 15 crabs which he kept in aquaria. From the available data he concludes that an egg hatched in June becomes a *megalops* by the latter part of July, passes through the first five post-larval instars during August, and four more during September and October. Growth from October until April ceases due to the low temperatures (it is supposed that crabs do not moult when the temperature is less than 60°F.) and the young crabs winter in the 9th or 10th instar. They are at this time 1.25 to 1.50 inches in width. Growth and moulting are resumed about the middle of April or the first of May and during the succeeding four months they moult five or six times. Sexual maturity is reached in July or August of the second season. This conclusion is supported by the fact that mating crabs are found at this season. No conclusive evidence bearing on the duration of life is available but it is supposed that the ordinary span of life is three years. No growth curve is presented for the Blue crab.

The Japanese King Crab, *Paralithodes camtschatica*

Marukawa (1933), in a paper on the Japanese King crab, presents a growth curve in which the males reach the maximum carapace width of 21 cm. in 30 years and the females 17 cm. in 24 years. Since this is the only growth curve, as far as can be ascertained, which has ever been presented for any of the larger crustaceans, it deserves careful analysis. It is particularly notable for the great age attributed to the larger crabs.

The data upon which Marukawa's growth curve is based are similar to those used in the present investigation; both are based on a study of size frequencies, the increase per moult, and the frequency of moulting. The size frequencies in the King crab study are based on adequate numbers; namely, 975 small immature crabs, 3,956 males and 1,759 females. In order to interpret the graphs which represent the size frequencies of these crabs, other data are necessary. For example, it should be known where and when the collections were made and how they were measured. Complete information is unfortunately lacking in both the English summary and the Japanese text. The latter states that the crabs were collected in the sea of Nemuro early in April but this statement may not apply to all the crabs measured. However, the measurements are recorded in millimetres on the graphs. The size frequencies are presented in three figures which represent the unsexed young up to 7.5 cm., the males and lastly the females above this size. In the graph of the young, seven modes are recognized and numbered

and in that for the males, additional modes up to a total of 31 at a carapace width of 21.6 cm. are indicated. In a later figure these modes are again represented together with the age, each mode being identified with a particular year group. In this manner the maximum age of 31 years supposedly has been determined. The number of moults intervening between these modes is indicated and a total of 56 to 61 moults is thought to occur during the lifetime of the animal. The females are treated in the same manner in other figures.

The estimation of age from size-frequency modes is a well-established method. It is, however, ordinarily difficult to recognize yearly modes except during early life. This is due to the overlapping of successive year groups resulting from variations in growth, and consequent obliteration of the modes. In no other species have the yearly modes been identified throughout a life of approximately 31 years and, in consequence, the interpretation should be scrutinized with care.

The modes for the larger crabs are very close together, the last four males and females being 2 mm. apart and on the even millimetres. Modes as little separated as these are, to say the least, highly improbable.

In another figure the percentage increase per moult for 75 specimens of both sexes up to a carapace width of 22 cm. is presented. The increase in crabs under 1 cm. in width is given as 30 per cent and with increasing size the per cent decreases. At about 7 cm. the sexes begin to differ, the female showing a smaller increase. Both sexes eventually fall to about 1 per cent. These increases are in general agreement with the size differences indicated by modes and with the number of moults assigned to each interval.

It will be evident from the foregoing account that Marukawa has presented an analysis carefully worked out, and internally consistent. However, in spite of these facts, it is believed that serious errors of interpretation have occurred, and that the assigned ages are four or five times too great. The increases per moult appear very low. The growth of *Cancer magister*, later to be presented, is based on over 600 moulting records, or more than eight times as many as were available for the Japanese King crab. In all these 600 records no moulting increase was found which was as low as 1 or 2 per cent., the lowest being 8 or 9 per cent. It must be admitted that these are different species, but for all other species for which data are available, namely, *Cancer pagurus*, *Cancer productus*, *Cancer gracilis*, *Hemigrapsus*, *Pachygrapsus*, *Homarus americanus* and *Callinectes sapidus*, very few records of increases as small as those given by Marukawa have been found. A possible serious source of error may have been overlooked by this investigator. It was found in the case of *Cancer magister* that crabs moulting in live-wells or laboratory aquaria gained considerably less per moult, especially in young crabs, than was the case in nature. Enough records of moults in nature were obtained to show this fact conclusively and an examination of the data on *Cancer pagurus* and *Cancer productus* indicates the probability of a similar situation in these species. In view of these facts it seems probable that Marukawa in his experimental results obtained a very small increase per moult and was misled by this to assign too many moults to the intervals between modes. Two interpretations are open: (1) the early modes may represent year groups, or

(2) they may represent instars. Thus he considers that between modes at 7, 17, 25, 34, 42, 53, 69, 85 and 101 mm. there intervene respectively 4 to 5, 2 to 3, 2, 2, 2, 2, and 2 moults. These modes may represent instars between which the per cent increases are 143, 47, 36, 23.5, 26, 30, 23 and 19 respectively. With the exception of the first interval (143 per cent), the modes correspond to single moults in *Cancer* rather than to the 2 to 5 moults per mode assigned by Marukawa.

In general it seems probable that the early modes represent instars rather than year groups and that the later modes merely indicate chance irregularities to which no statistical significance may be attached.

The European Edible Crab, Cancer pagurus

Williamson (1904) and Pearson (1908) have studied the growth of *Cancer pagurus* and have obtained certain interesting moulting records which agree closely with those obtained for *Cancer magister* under laboratory conditions. Williamson obtained records of 14 crabs which moulted in the Marine Laboratory, Bay of Nigg, Scotland, and he reports also the results obtained by Waddington in raising three crabs through 12 instars, 7 instars and 4 instars, respectively. Peason's results are also partly based upon 17 moulted crabs which he measured at the Nordsee Museum, Helgoland.

Pearson, in interpreting these rather limited data, makes the serious error of averaging all the size increases occurring at moulting. He overlooks the fact that even in his own data the increase per moult decreases with increasing body size. It is apparently assumed that individual differences at ecdysis are random and average about 25 per cent. On this basis the sizes at each year for nine years have been calculated. The effects of the error just mentioned are cumulative and consequently large for the later instars. Moreover, factual data on the intervals between moults are largely lacking and the calculations based on this point are mere speculation. In view of these facts Pearson's conclusions on the growth-rate of *Cancer pagurus* are unsatisfactory and they fail to represent either natural growth or growth under laboratory conditions.

GROWTH OF CANCER MAGISTER

Collection of the Data

Most of the measurements used in the present work were secured in Boundary bay, British Columbia, and cover a period of approximately 20 years. The data comprise upward of 20,000 measurements.

All measurements were made with sliding jaw calipers reading directly to millimetres and by a vernier to tenths of a millimetre. The width of the carapace was measured from tip to tip of the tenth anterolateral teeth on either side, thus giving the maximum width, and the length in the midline from the tip of the rostrum to the transverse line of granules near the posterior margin of the carapace.

MOULTING RECORDS

Realizing the importance of direct information on the moulting increase of male and female crabs at various sizes, intensive efforts were made to collect

such data. Compartment live-wells were constructed and anchored to a float belonging to the Crescent Oyster Company and situated at the head of Boundary bay. The live-wells were placed about one foot (30 cm.) beneath the surface of the water in a position in which they were ordinarily shaded from above. The use of coarse screening permitted the free passage of water through the compartments.

The larval and post-larval crabs, which could not be kept in this way, were placed in individual dishes in the laboratory. They were provided with clean sand, and fresh food. The water was frequently changed and care was taken to keep the water temperature as close as possible to that of the bay.

The crabs used in the moulting experiment were procured, for the most part, from frequent and intensive examination of the shore at low tide. A few were obtained during commercial crab-fishing. Large numbers of crabs were found buried in the sand near low-tide mark; many of these were on the point of moulting and some shed their shells within a few hours of capture. The compartment live-wells provided accommodation for nearly 200 crabs and the compartments were ordinarily kept fully occupied. Preference was always given to those crabs which it was thought would moult first; other crabs were often liberated to make room for those showing signs of approaching ecdysis. In this manner the period in captivity was kept at a minimum, thus reducing the error which is apt to result from this source.

While the moulting experiment was in progress during the summers of 1932 and 1933, every attempt was being made to procure measurements of crabs which had moulted under absolutely natural conditions. For this purpose, the beaches were continually searched at low tides and upwards of 30 crabs were found, with their cast shells. These crabs were ordinarily found under inverted cockle shells at very low tides. In each case the crab and its cast shell were checked for size, sex, and markings and the records from this source were only used when identification was certain. These data, though small in numbers, constitute a valuable check against the results from moulting in the laboratory and in live-wells.

The moulting measurements collected by the second author were procured in earlier years in the same locality in essentially the same manner as those described above. The data from these two studies are all that are known to have been recorded for this species. The combined records comprise measurements of the size both before and after moulting for more than 600 crabs. This, it should be pointed out, is more than three times as many records as are comprised in all other previous crab studies combined. In addition to these data, size frequencies, based on over 5,000 larval and post-larval crabs, give comparable data on the moulting increase during the early instars.

LIVE CRABS

Numerous collections of live crabs were made with the use of traps covered with fine-meshed netting, by dredging, and by collecting by hand in shallow water. Several thousand crabs were collected in these ways and the measurements of several thousand others were secured from the commercial catch.

EXOSKELETONS

In addition to the live crabs, a very thorough search was made almost daily for the exoskeletons which are cast during ecdysis. During the seasons of 1932-1933, more than 8,000 of these were obtained and measured. Cast shells were scarce during the final week of March and the month of April, the earliest part of the season during which field work was carried on, and more abundant later. The height of the moulting season as judged by the abundance of exoskeletons was probably in June after which month a steady decline was noted. The exoskeletons were gathered frequently over several miles of shoreline and were not selected as to size or sex.

Disintegration-rate of exoskeletons. In a study involving the partial determination of growth-rate by means of exoskeletons, it is obviously desirable to know the probable age of these shells or, in other words, to know how soon they will fall to pieces when exposed to the elements. Several pieces of evidence bearing on this lead to the assumption that disintegration is rapid.

The cast shells of three crabs which moulted during the night of June 9 were placed in the open on the morning of June 10. By June 14, five days later, two of these were so seriously cracked that, if found on the beach, they would not have been measured. The weather at this time was very warm. Another collecttion of exoskeletons, placed between high- and low-tide marks in front of the laboratory fell to pieces in less than a week. From these observations it would appear that the numerous cast shells used in the study were not the accumulation of years or of months, but rather of days. Moreover, since the collections were made on the same stretch of beach day after day, none but the first collections could possibly have been the accumulation of a long period.

Interpretation of the Results

Having seen the source of the data we may now turn to the interpretation of the figures obtained, but first it will be well to present the early life-history by way of orientation. Mating occurs in May or June and the ovarian eggs reach a size of slightly less than half a millimetre by the time of laying, which occurs in October or November. The eggs are carried by the female attached to the abdominal pleopods and hatch in the spring of the following year. Appearing as *protozoea* they pass through the *zoeal* and *megalops* instars. By June or approximately one year after mating, the larval life is completed and the first postlarval young are found. We will next attempt an analysis of the growth of these postlarval crabs, considering first the increase per moult, next the time relations of the moults, and last the synthesis of these facts into a growth curve.

INCREASE IN CARAPACE WIDTH PER MOULT

In the statistical analysis of the size increase at ecdysis, only records of crabs moulting successfuly were considered. All the records were grouped according to the size before moulting. For crabs under one centimetre in carapace width, they were grouped according to instar size (the early instar sizes were known

from other data presented elsewhere in this paper). For all crabs above one centimetre in width they were grouped by one centimetre intervals. For example, all crabs between 5.00 and 5.99 cm. inclusive were grouped together and tabulated according to the per cent increase. At first male and female measurements were separately tabulated and analyzed but, since no differences in increase could be detected for crabs under 10 cm., the sexes up to this size were later combined.

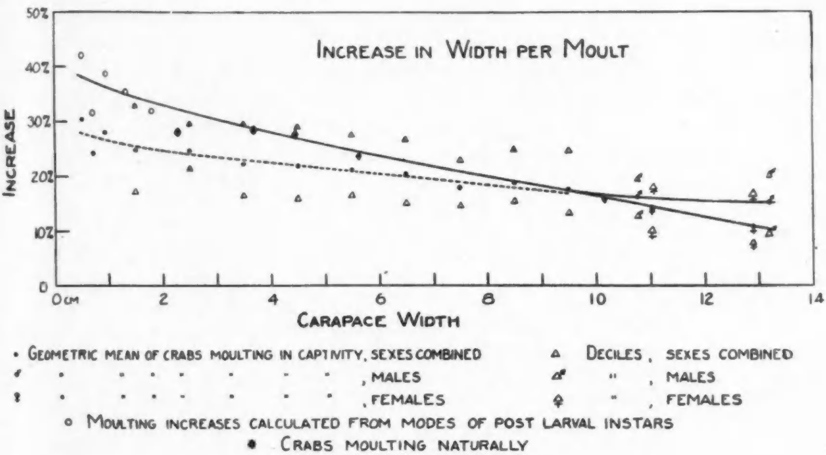


FIGURE 1. Increase in width per moult. Broken line fitted to geometric means of combined sexes below a carapace width of 10 cm.; solid line fitted by eye to moulting increases calculated from modes of post-larval instar sizes (open circles) and natural moultings (solid diamonds).

The per cent increase obtained from crabs moulting in captivity is given in table I and is indicated by the broken line in figure 1. The solid line represents the most probable natural increase based first on the records of moulting in nature (squares), second on the increase from instar to instar (open circles), and on the data just mentioned as obtained from crabs moulting in captivity. It should be noted that despite efforts to approximate natural conditions, the increase of crabs in live boxes was only about three-fourths of the normal among the smaller individuals. Unfortunately there are little data for natural moulting among mature crabs, but the data at hand indicate that the increase does not differ significantly from that in captivity.

These moulting records indicate that the per cent increase per moult decreases from nearly 40 per cent at 0.5 cm. to about 15 per cent in males of 13.5 cm. and to about 10 per cent in females of about 13.0 cm. Males and females apparently increase at the same rate until they attain a carapace width of about 10.0 cm., the size at which females become mature. Thereafter the

males increase more rapidly than the females. These increases are difficult to reconcile with the theoretical increase of 26 per cent advocated by Przibram. For example, a linear increase of 10 per cent results in a mass increase of 33 per cent; one of 15 per cent in a mass increase of 50 per cent; one of 30 per cent in a mass increase of 119 per cent, and one of 35 per cent in a mass increase of 150 per cent.

Frequency distributions for the sizes before and after ecdysis are given in figure 2 for crabs moulting during August and September.

By way of comparison with *Cancer magister*, it may be noted that *Cancer productus*, kept under identical conditions, showed a similar retardation during the early instars. Likewise the data on the moulting of *Cancer pagurus* (Williamson 1904; Pearson 1908) fit the curve derived from the experimental data very closely. It would appear therefore that the growth of the European edible crab is essentially similar to that of the Pacific edible crab.

TABLE I. Moulting increase

Carapace width (mid-values of classes in cm.)	Sex	Number	Per cent. increase	1st decile (per cent)	9th decile (per cent)
		From Modes of Frequency Distributions			
.52	Combined		42.307		
.74	Combined		31.081		
.97	Combined		38.144		
1.34	Combined		35.821		
1.82	Combined		31.868		
2.40	Combined				
		Experimental Moulting			
			Geometric mean		
.52	Combined	29	30.328	21.9	43.1
.72	Combined	100	24.087	16.0	32.0
.94	Combined	10	27.924	23.0	30.3
1.50	Combined	22	24.763	17.1	32.8
2.50	Combined	3	24.784	21.3	29.7
3.50	Combined	39	22.168	16.4	29.5
4.50	Combined	105	21.862	15.8	28.9
5.50	Combined	65	21.041	16.5	27.5
6.50	Combined	49	20.283	14.9	26.5
7.50	Combined	36	17.832	14.5	22.9
8.50	Combined	16	18.673	15.3	24.7
9.50	Combined	20	17.408	13.0	24.5
11.00	Male	21	16.110	12.5	19.4
11.00	Female	11	14.129	10.0	18.4
14.00	Male	9	15.247	9.3	20.1
13.00	Female	8	10.887	7.8	16.7
		Natural Moulting			
2.29	Female	1	28.000		
3.69	Combined	4	28.492		
4.46	Combined	13	27.860		
5.63	Combined	2	23.530		
10.15	Male	2	15.960		

SIZE FREQUENCY

Most species of animals have a characteristic annual spawning season. As a consequence the young of one year ordinarily have grown sufficiently by the next spawning season to be distinguished from the preceding brood. Statistically this means that the two age groups would be clearly indicated by two modes in a frequency distribution based on size. In this manner it is sometimes possible to determine several year groups, but no investigator has been able to follow growth for 30 years by this method as Marukawa (1933) has attempted to do.

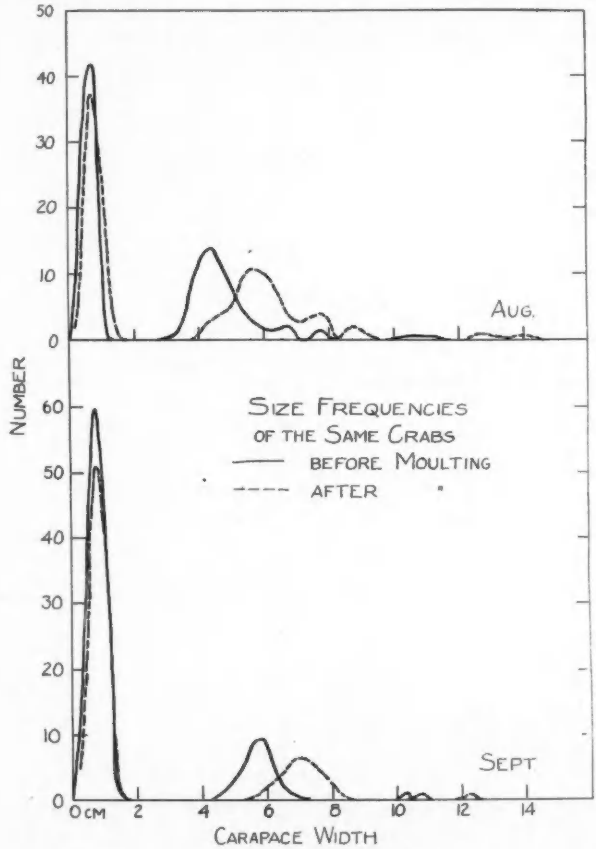


FIGURE 2. Size frequency distribution of the same crabs before and after moulting; sexes not separated.

The size frequencies of live crabs and of exoskeletons (the latter should be smaller by one moult than the live crabs taken at the same time) are shown in figure 3. Here the composition of the population from May to September is

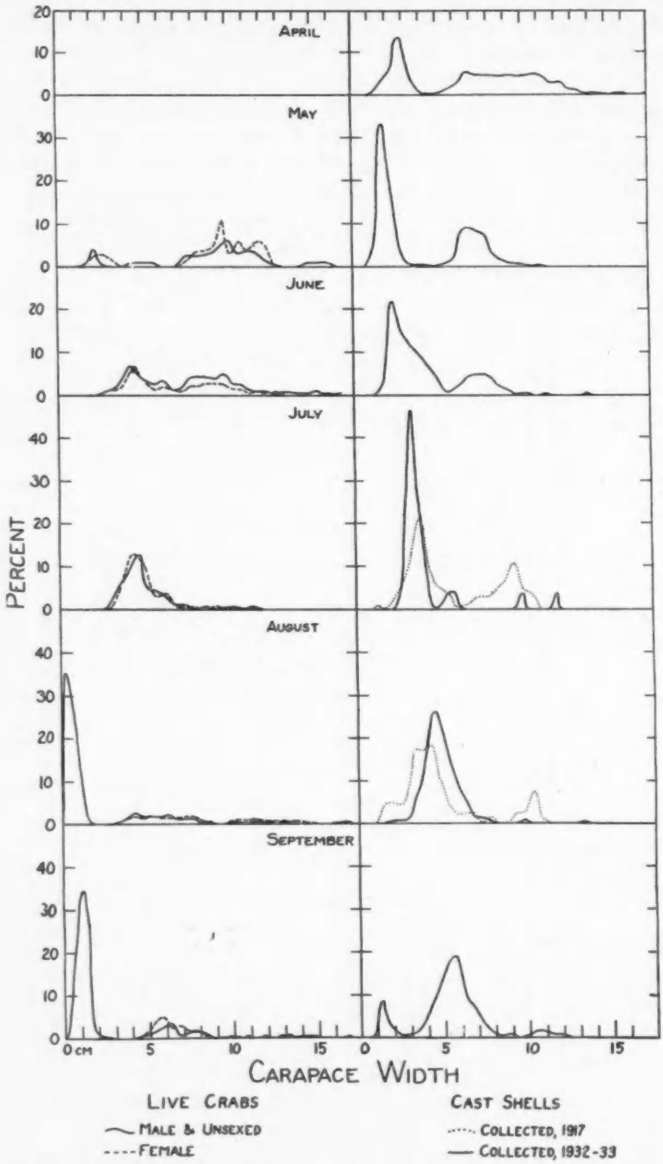


FIGURE 3. Size frequency distribution of live crabs (left column) and of cast shells (right column). Numbers expressed as percentages. Collected in 1931-32 except as indicated for 1917.

represented, the numbers being expressed in percentages. It will be noted that, for example in September, three year groups may be distinguished; the young of the year at 1 or 2 cm., of the preceding year at 4 to 7 cm. and of the second season previous, less clearly, at 10 to 12 cm. The increase in size of the young of the preceding year, here the most numerous class, may readily be followed from month to month, and the appearance of the young of the year noted in August.

TABLE II. Size-frequencies of 5,065 crabs, up to and including 3.00 cm. carapace width

Width in cm.*	Number	Width in cm.	Number	Width in cm.	Number	Width in cm.	Number
0.01		0.41		0.81	19	1.21	10
.02		.42		.82	19	1.22	2
.03		.43		.83	11	1.23	20
.04		.44	1	.84	17	1.24	11
.05		.45	2	.85	18	1.25	16
.06		.46	2	.86	21	1.26	10
.07		.47	13	.87	19	1.27	12
.08		.48	15	.88	19	1.28	17
.09		.49	45	.89	24	1.29	7
.10		.50	59	.90	38	1.30	11
.11		.51	68	.91	48	1.31	6
.12		.52	67	.92	49	1.32	10
.13		.53	76	.93	42	1.33	17
.14		.54	58	.94	54	1.34	13
.15		.55	35	.95	54	1.35	16
.16		.56	15	.96	52	1.36	9
.17		.57	10	.97	37	1.37	11
.18		.58	6	.98	40	1.38	12
.19		.59	3	.99	36	1.39	13
.20		.60	2	1.00	38	1.40	11
.21	1	.61	1	1.01	29	1.41	7
.22	1	.62	4	1.02	20	1.42	8
.23	8	.63	5	1.03	25	1.43	14
.24	6	.64	5	1.04	28	1.44	8
.25	20	.65	9	1.05	29	1.45	8
.26	21	.66	8	1.06	17	1.46	9
.27	38	.67	22	1.07	13	1.47	15
.28	37	.68	45	1.08	11	1.48	7
.29	27	.69	39	1.09	9	1.49	10
.30	39	.70	45	1.10	15	1.50	14
.31	18	.71	53	1.11	4	1.51	5
.32	7	.72	72	1.12	7	1.52	5
.33	3	.73	107	1.13	13	1.53	7
.34	1	.74	97	1.14	7	1.54	1
.35		.75	54	1.15	3	1.55	7
.36		.76	73	1.16	7	1.56	6
.37		.77	69	1.17	9	1.57	4
.38		.78	45	1.18	10	1.58	5

*Recorded to nearest hundredth.

TABLE II.—*Continued*

Width in cm.*	Number	Width in cm.	Number	Width in cm.	Number	Width in cm.	Number
.39		.79	40	1.19	7	1.59	7
.40		.80	23	1.20	11	1.60	3
1.61	3	2.01	12	2.41	26	2.81	6
1.62	7	2.02	22	2.42	23	2.82	14
1.63	3	2.03	15	2.43	26	2.83	7
1.64	3	2.04	14	2.44	21	2.84	9
1.65	5	2.05	13	2.45	10	2.85	7
1.66	4	2.06	15	2.46	18	2.86	10
1.67	6	2.07	18	2.47	18	2.87	11
1.68	16	2.08	12	2.48	14	2.88	12
1.69	11	2.09	11	2.49	22	2.89	12
1.70	9	2.10	15	2.50	27	2.90	13
1.71	10	2.11	5	2.51	21	2.91	7
1.72	13	2.12	9	2.52	21	2.92	10
1.73	12	2.13	13	2.53	22	2.93	9
1.74	13	2.14	25	2.54	23	2.94	10
1.75	11	2.15	13	2.55	23	2.95	6
1.76	11	2.16	16	2.56	23	2.96	6
1.77	17	2.17	17	2.57	19	2.97	8
1.78	21	2.18	13	2.58	17	2.98	8
1.79	22	2.19	16	2.59	17	2.99	9
1.80	34	2.20	26	2.60	25	3.00	13
1.81	15	2.21	11	2.61	15		
1.82	24	2.22	22	2.62	15		
1.83	23	2.23	18	2.63	11		
1.84	21	2.24	17	2.64	16		
1.85	29	2.25	24	2.65	16		
1.86	19	2.26	17	2.66	8		
1.87	34	2.27	16	2.67	12		
1.88	26	2.28	26	2.68	11		
1.89	11	2.29	19	2.69	13		
1.90	31	2.30	16	2.70	12		
1.91	31	2.31	16	2.71	5		
1.92	21	2.32	28	2.72	10		
1.93	12	2.33	17	2.73	9		
1.94	19	2.34	24	2.74	12		
1.95	13	2.35	33	2.75	14		
1.96	21	2.36	26	2.76	4		
1.97	22	2.37	28	2.77	10		
1.98	22	2.38	31	2.78	17		
1.99	13	2.39	16	2.79	7		
2.00	21	2.40	29	2.80	9		

*Recorded to nearest hundredth.

The determination of instar sizes from frequency distributions involves essentially the same method as that described above. It is, however, a method limited to animals which display saltatory growth or increase by "steps". As a

consequence it has seldom been used in the analysis of life-histories. In this method time is left out of consideration. Assuming that all crabs hatch from eggs of approximately the same size and that growth proceeds in an orderly fashion, the early post-larval instars should show a certain degree of similarity in size. This similarity of size will gradually disappear due in part to the many factors of the environment such as food, temperature, and salinity, which influence growth and which may have an unequal influence on different individuals. However, it may be possible to identify several instars from a frequency distribution, each instar being represented by a mode which approximates a "normal curve".

In table II and figure 4 the size frequencies of 5,065 crabs up to, and including, 3.00 cm. in carapace width are presented. In the graph four modes are clearly shown and three others indicated. Beyond this point the plotted data failed to show significant modes and therefore have not been reproduced here. The *zoeal* instars are not indicated and the first mode in this figure represents the *megalops*. The succeeding modes have been taken to indicate the early post-larval instars. It should be pointed out here that, due to the scale used in figure 3, the instar modes do not appear there.

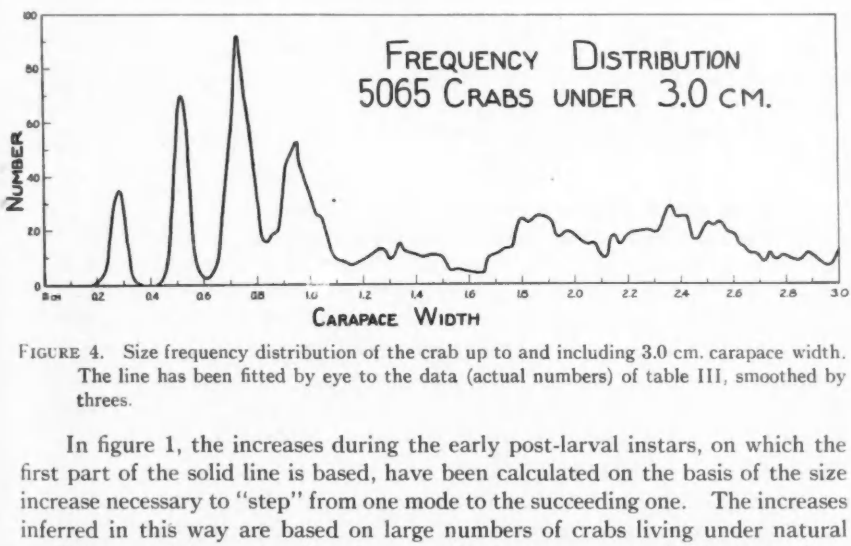


Figure 4. Size frequency distribution of the crab up to and including 3.0 cm. carapace width. The line has been fitted by eye to the data (actual numbers) of table III, smoothed by threes.

In figure 1, the increases during the early post-larval instars, on which the first part of the solid line is based, have been calculated on the basis of the size increase necessary to "step" from one mode to the succeeding one. The increases inferred in this way are based on large numbers of crabs living under natural conditions and have therefore been emphasized as more reliable than those obtained in the laboratory or live-wells.

Contrasted with the natural increases just mentioned, those of the experimental crabs are definitely retarded. This is clearly shown in figure 1 and it will be further observed that the large crabs appear to have suffered less from the experimental conditions than the small crabs. This is to be expected since the small crabs were kept in the laboratory and the large crabs in anchored live-wells.

The second and subsequent moults, when they occurred in the laboratory, were clearly below the increase shown by crabs of corresponding size at the first moult under laboratory conditions. This evidently shows the cumulative effect of the unfavourable conditions of confinement.

That the modes indicated in figure 4 represent actual instars is shown by moulting records for small crabs which passed through one or more instars in the laboratory. In this manner, though the increases admittedly were smaller, it has been possible to check the sizes so there can be no doubt that the first 4 modes represent instars.

COMPENSATORY GROWTH

One factor which contributes to the distinctness of the early modes is an unusual compensatory moulting-increase in the early instars. A comparison of the sizes of *magalops* with the first post-larval crabs, into which they moult, shows little or no correlation ($r = -0.132$), all sizes of *megalops* giving rise to crabs of approximately the same width. When the percentage increase is plotted against the *magalops* size a marked negative correlation ($r = -0.762$) is found, the small larvae showing a large percentage increase and vice versa. As a result, the size of the first post-larval instar is uniform and the variability is small. This is the clearest example of compensatory growth that has come under our notice; its concentration, so to speak, in a single moult serves to make it very striking. The uniformity of size produced in the first post-larval instar by this wiping out of the dispersion of the *megalops* is apparent in subsequent instars, and in consequence the modes may be recognized for a much longer time than would otherwise be possible.

THE INTERVAL BETWEEN MOULTS

Earlier and less complete data indicated the average duration to be 11.4, 11.6, 10.0, 21.6 and 34.3 days, respectively, for the first to the fifth post-larval instar. One crab spent 31 days in the third instar.

The evidence from tagging experiments is purely negative in character. If a crab should be recovered two years after liberation it would then be clear that it had not moulted in the meantime; otherwise the identifying tag would have been lost. In the event that many crabs should be tagged and none recovered beyond a certain period, this would constitute good negative evidence that moulting had occurred and all tags had been shed.

Approximately 1,100 large crabs of both sexes were tagged by the first author in northern British Columbia and in Boundary bay, and several hundred others were tagged by the second author at San Francisco and by Spencer (1932) in Clayoquot sound, Vancouver island. In only one case was there clear evidence that more than one year had elapsed without moulting. Since returns came in for a time but not in sufficient numbers to reduce greatly the number still at large, later returns should have continued unless prevented by the occurrence of moulting. Hence the evidence from tagging, though not conclusive, favours the theory of annual moulting in the larger crabs.

NUMBER OF INSTARS

The determination of the early instars from the modes of frequency distribution checked against actual individual records has been outlined (see also figure 4). The per cent increase for males and females according to size has been discussed and the results are graphically shown in figure 1, in which the solid line indicates what are believed to be the natural increases and the dotted line the experimental results.

TABLE III. Instar sizes

Instar no.	Sex	Instar size in cm.	Per cent increase
Megalops	Combined	.28*	
1st P. L.	"	.52	42.3
2nd P. L.	"	.74	31.1
3rd P. L.	"	.97	38.1
4th P. L.	"	1.34	35.8
5th P. L.	"	1.82	31.9
6th P. L.	"	2.40	31.2
7th P. L.	"	3.15	30.2
8th P. L.	"	4.10	28.0
9th P. L.	"	5.25	25.1
10th P. L.	"	6.57	22.5
11th P. L.	"	8.05	19.0
12th P. L.	"	9.58	17.5
13th P. L.	Male	11.26	15.4
13th P. L.	Female	11.26	13.0
14th P. L.	Male	13.00	15.1
14th P. L.	Female	12.73	11.0
15th P. L.	Male	14.96	14.2
15th P. L.	Female	14.13	8.6
16th P. L.	Male	17.08	11.3
16th P. L.	Female	15.35	
17th P. L.	Male	19.01	

**Megalops* and first six post-larval instars are determined from the size frequency distribution (figure 4). Subsequent values estimated from per cent increase per moult (table I).

The number of post-larval instars has been calculated from these two sets of data. Knowing the sizes of the early instars and the increases to be expected, the approximate sizes of the later stages have been calculated and are presented in table III; they are graphically represented in figure 5. In this figure, carapace width is plotted against instar number. The resultant curve is concave upward and increases in slope with the higher instars. At about 10 cm. the lines for the sexes separate; the females at the later instars are smaller than the corresponding males. In order to reach the maximum size attained by males and females in Boundary bay, 17 and 16 post-larval instars respectively, would be required. The time relationships, while partly indicated in figure 5, will be discussed later.

SEASONAL OCCURRENCE OF THE VARIOUS INSTARS

Considering the size-frequencies of crabs taken at different seasons from the point of view of the instars indicated in table III and figure 5, the following results have been obtained:

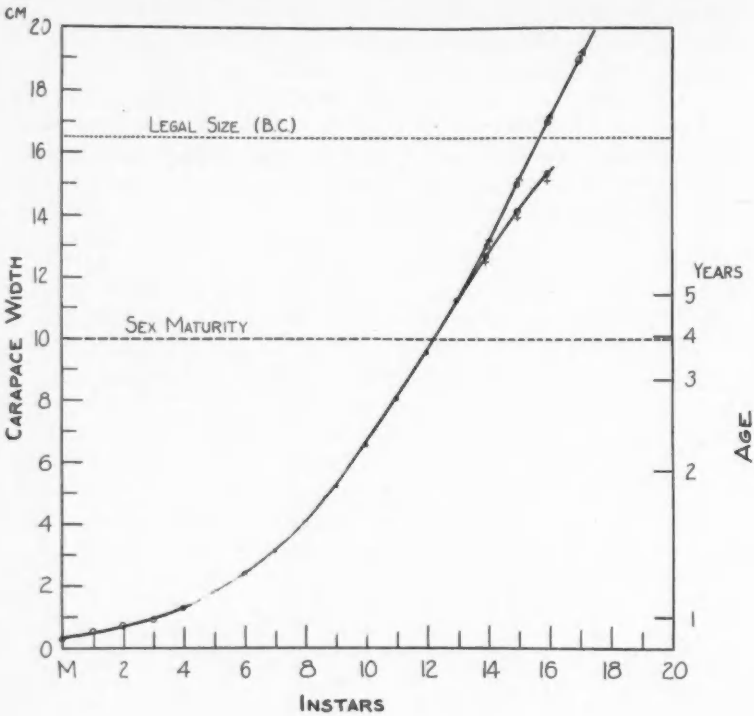


FIGURE 5. Instar sizes from data in table IV. The first seven points are from modes of the size frequency distribution (fig. 4); the subsequent ones are estimated from moulting increases shown in figure 1 and table I.

Instar	Predominately present	Present to a lesser degree
Megalops	July, August	September
1st post-larval	August	September
2nd " "	August, September	
3rd " "	September	October, November
4th " "		September
5th " "		
6th " "	May	
7th " "	June, July	May, August
8th " "	June, July, August	September

9th post-larval	July, August, September
10th " "	August, September
11th " "	May, June
12th " "	May, June

GROWTH-RATE

From an analysis of the size increases of males and females at moulting, the predominant sizes of both live crabs and exoskeletons at various times of year, as well as the available data on moulting frequency, the growth curve in figure 6 has been constructed. The central heavy line indicates what is thought to be the average growth-rate and the lighter lines indicate the probable size-limits at corresponding ages. For widths above 10 cm. the sexes differ and here the males are represented by a dashed, the females by a dotted line. It will be noted that, in the older crabs, the males exceed in size the females of the same age.

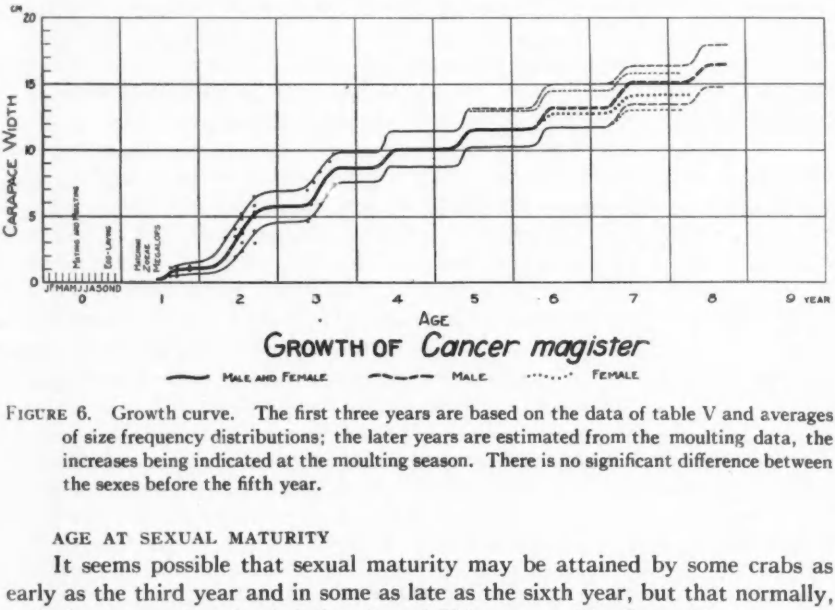


FIGURE 6. Growth curve. The first three years are based on the data of table V and averages of size frequency distributions; the later years are estimated from the moulting data, the increases being indicated at the moulting season. There is no significant difference between the sexes before the fifth year.

AGE AT SEXUAL MATURITY

It seems possible that sexual maturity may be attained by some crabs as early as the third year and in some as late as the sixth year, but that normally, crabs become mature in their fourth and fifth years. (See figure 6.)

AGE AT LEGAL SIZE

Legal size in British Columbia is 6½ inches or 16.5 cm., a size which is probably attained during the seventh or eighth year. Very few females ever reach this size.

MAXIMUM AGE

Allowing for one or two years during which crabs possibly do not moult, though there is no conclusive evidence that this often happens, the maximum age

of the species would be ten years. Eight years probably represents the average duration of life in Boundary bay. If the larger size observed at Prince Rupert and other northern points is not merely the result of a less intense fishery, it may indicate a greater age for the colder northern waters. This would be in harmony with the greater size and age observed in the north in the case of some marine forms having an extensive range in latitude such as the razor clam, *Siliqua patula* (Weymouth, McMillan and Rich 1931).

Figure 6 represents a synthesis of all the available data which bear on the growth of *Cancer magister* and this figure represents the first growth curve for this species.

DISCUSSION

Previous attempts to determine the growth of Brachyura have been unsatisfactory for several reasons. The data have been insufficient for reliable statistical treatment throughout the size range of the species; moulting records, because of their paucity, could not be analyzed to show the fundamental decline of moulting increase with age, but have merely been averaged; for similar reasons the greatly reduced gains of crabs moulting in captivity as compared with the natural process have not been recognized; by some workers the modes of frequency distributions which represent instars have been misread as year classes.

The present data show conclusively that the increase per moult declines throughout life in *Cancer magister*. This is supported by the data of Marukawa on the moulting of the Japanese king crab, *Paralithodes camtschatica*. That the other factor of growth, interval between moults, also changes with age is clear from various data. For example, the data of Nicholls (1931) on the isopod *Ligia* show not only that the increase per moult falls with increasing size but that the interval between moults increases with age, both factors thus contributing to slow growth. That the growth-rate for animals in general falls with age, was early pointed out by Minot (1891) and has been emphasized by the second author (1931). Growth in the Crustacea apparently conforms to this general rule, although the fact has not been generally recognized by previous workers.

The view of Przibram and others that arthropods double in weight and increase 26 per cent in linear dimensions at each moult thus fails in the case of *Cancer magister* and, as far as we have data, in other crabs and probably in all Crustacea. No data on insects are here considered.

SUMMARY

Data are here presented on the growth of *Cancer magister*, the Pacific edible crab. The conclusions are based on three types of evidence, moulting (increase per moult, and interval between moults), tagging, and size frequencies. These data have been collected over a period of almost 20 years and comprise approximately 20,000 measurements.

Part of the measurements (more than 8,000) were obtained from moulted exoskeletons. That these are satisfactory for use in the analysis of growth is shown by the fact that they disintegrate rapidly on exposure; the shells collected therefore represent current conditions.

Moulting records indicate that the per cent increase per moult decreases from nearly 40 per cent at 0.5 cm. to about 15 per cent in males at 13.5 cm. and 10 per cent in females at 13.0 cm. Males and females apparently increase at the same rate up to a carapace width of about 10 cm. and thereafter the males increase more per moult than the females.

Experimental records plainly indicate that the increase per moult is less in live-wells and aquaria than under perfectly natural conditions. The second and third moults, when they occur in the laboratory, are clearly below the increase in crabs of the same size at the first moult in the laboratory. This clearly shows the cumulative effect of confinement.

The sizes of 5,065 small crabs under 3 cm. plotted in the form of a frequency distribution, indicate 7 modes, each taken to represent an instar. In this manner, the natural increases per moult in early life have been ascertained. These conclusions have been checked against crabs which moulted in the laboratory where the number of instars was known.

The intervals between the instars increase with increasing age. Thus, during the first five post-larval instars, an increase from 11 days to 34 days has been observed under laboratory conditions.

Evidence from tagging experiments supports the conclusion that the majority of large crabs moult yearly. Intervals of two years between moults, if they occur, must be extremely rare.

It has been possible to calculate the number of instars by making use of the average sizes in the early instars and the percentage increase per moult. In order to reach the maximum size attained by males and females in southern British Columbia, 17 and 16 post-larval instars would be required respectively.

Megalops appear during July and August; 1st post-larval crabs during August; 2nd post-larval crabs during August and September; 3rd during September; 6th during the following May; 7th during June and July; 8th during June, July and August; 9th during July, August and September; 10th during August and September; 11th and 12th during the following May and June.

It seems probable that sexual maturity in female crabs is ordinarily attained during the fourth or fifth year, but that it may come as early as the third year or as late as the sixth year.

The legal size in British Columbia, 6½ inches or 16.5 cm., is probably attained during the seventh or eighth year. Very few females ever reach this size.

The average duration of life in southern British Columbia is probably about 8 years. Allowing for individual differences, it is possible that some crabs may reach an age of 10 years.

ACKNOWLEDGEMENTS

The writers wish to acknolwedge the assistance of Dr. Willis H. Rich, of Stanford University, who has followed the entire work and given valuable advice in the interpretation of data. Information was first collected for the British Columbia Provincial Fisheries Department (by F.W.W. in 1914 and following summers) and much of this is now utilized for the first time. We wish to record

our indebtedness to Mr. John P. Babcock, at that time Assistant to the Commissioner of Fisheries, at whose suggestion the work was undertaken. Most of the data were gathered for the Biological Board of Canada (by D.C.G.M. from 1930 to 1934). We desire to express to Dr. W. A. Clemens, Director of the Pacific Biological Station, our appreciation of his understanding interest in, and active support of this investigation.

REFERENCES

BAUMBERGER, J. P. AND J. M. D. OLMSTEAD. *Physiol. Zool.* **1**, 531-549. 1928.

CALVERT, PHILIP P. *Proc. Amer. Philosoph. Soc.* **68** (3). 1929.

CHURCHILL, E. P. *Bull. U.S. Bur. Fish.* **36**, 95-128. 1919.

HERRICK, F. H. *Bull. U.S. Bur. Fish.*, **29**, 1909, 149-418. Doc. 747. 1911.

MARUKAWA, H. *J. Imp. Exper. Sta. Tokio* **4** (37). 1933.

MINOT, C. S. *J. Physiol.*, **12**, 147. 1891.

NICHOLLS, A. G. *J. Mar. Biol. Ass. N.S.* **17** (3), 655-705. 1931.

PEARSON, J. Cancer. L.M.B.S. Memoirs on typical British marine plants and animals. London. 1908.

PRZIBRAM, H. Connecting laws in animal morphology. London University Press. 1930.

SPENCER, G. J. *Bull. Biol. Bd. Can.* **30**. 1932.

WEYMOUTH, F. W., H. C. MCMILLAN AND WILLIS H. RICH. *J. Exper. Biol.* **8**, 228-249. 1931.

WILLIAMSON, H. C. *Ann. Rep. Fish. Bd. Scot.* **22**, 100. 1904.

Local Differences in the Body Proportions of the Lobster, *Homarus americanus*

By W. TEMPLEMAN
McGill University

(*Received for publication September 15, 1934*)

ABSTRACT

The claws of males and the width and depth of abdomen of females increase at a higher rate than body length with approaching sexual maturity. Consequently for lobsters over 20 cm. in length males possess claws relatively larger and females an abdomen relatively wider and deeper in an area such as that near Pointe du Chêne where sexual maturity occurs at about 20 cm. than in that near Grand Manan where lobsters only become sexually mature at about 34 cm.

INTRODUCTION

Many workers on decapod Crustacea have noted a relation between sexual maturity and the size of the claw in male and the width of the abdomen in female decapods. Thus, according to Huxley (1932),

"the chela of the male fiddler crab (*Uca pugnax*) shows a decrease in its growth coefficient apparently at the time of sexual maturity while in the spider crab (*Maia squinado*) presumably after sexual maturity, both chelae begin to grow faster than the body, this heterogonic growth continuing until death. In the female Brachyura the abdomen is always heterogonic in some part of its development since it is always relatively narrow in the immature stages and relatively broad in the adult."

In the American lobster, Herrick (1911) says that the abdomen of the female lobster is relatively broader than that of the male, while the male has slightly larger claws. However, no detailed analysis of the relative proportions of various parts of the body in male and female lobsters of different sizes has hitherto been made, nor have we been able to find reference to studies of variation in relative body proportions of any decapods, due to environmental differences.

During 1932 a comparison was made of the relative proportions of various parts of male and female lobsters caught at Pointe du Chêne, cape Tormentine, Canso, and at Seal cove, Grand Manan. Attention was concentrated on variations in the length of the large claws and in the width and depth of the abdomen. The claw measurements were taken with dividers from the tip to the point of articulation with the 5th segment of the arm as shown in figure 1. The widths of the 2nd and 6th segments of the abdomen were measured with callipers across the ventral surface between the spurs which form the deepest points of the pleura.

J. BIOL. BD. CAN. 1 (3) 1935.

In measuring the depth of the abdomen, the lobster was laid flat on the measuring board, with ventral surface upward and abdomen extended, the vertical depth of the second segment of the abdomen being taken from the board to the top of the spur forming the deepest part of the pleura. The total length was measured from the tip of the rostrum to the end of the telson, not including the setae on the telson. All measurements were of live lobsters. Except in the case of six egg-bearing lobsters, 34.6 to 40.5 cm. in length, included in the Grand Manan graphs,

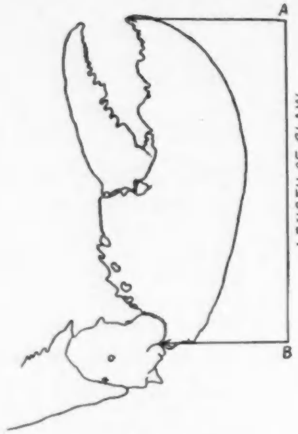


FIGURE 1. Dorsal view of lobster claw showing position of points used in measurements of length (after Herrick 1911).

all measurements of females are of individuals without eggs. Each graph to represent the growth in length of a part in relation to the total length of the animal was drawn separately, and one superimposed upon the other. Each point on a graph represents the measurement of a single lobster.

VARIATIONS IN MALES AND FEMALES

Figures 2 and 3 show the relative lengths of the crushing and the biting claws in male and female lobsters from Pointe du Chêne, Canso, and from Seal cove, Grand Manan. In all three localities the length of the biting claw relative to the total length remains the same at all sizes in the female and there is no significant difference between the relative lengths of the female claws in the above regions. In the male, however, after an early period during which its relative size remains constant, the claw begins to increase in length much more rapidly than the body, this increase continuing at even the largest sizes obtained. Even at the smallest commercial sizes, however, the claws of the male are relatively longer than those of the female.

In figure 4, showing the relative width of the second segment of the abdomen in male and female lobsters from the same three localities, the relative width of the abdomen of the male at Canso and Pointe du Chêne is slightly greater at the

larger than at the smaller sizes, while at Grand Manan this difference is not so apparent. The abdomen of the female, even at the smaller commercial sizes, is relatively wider than that of the male. At these small sizes the relative width of the abdomen of the female remains constant, while at larger sizes there is a gradual

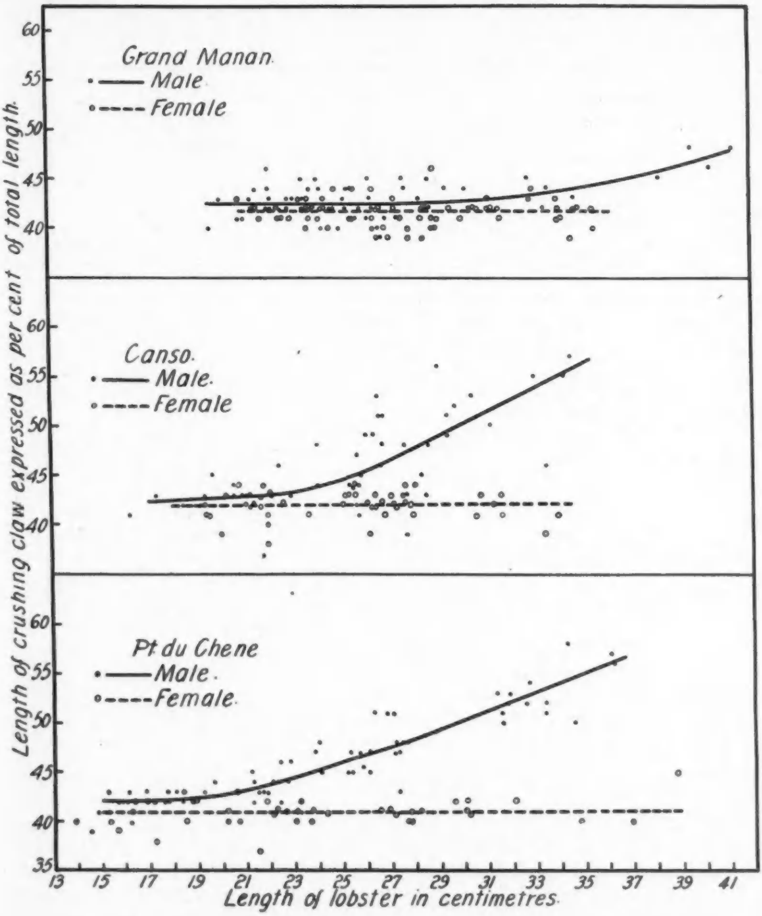


FIGURE 2. Increase in relative length of crushing claw with increase in total length of male and female lobsters at Grand Manan, Canso and Pointe du Chêne, 1932.

increase in the relative width. As size increases still further, the increase in relative width, although still continuing, becomes much less rapid.

In figure 5 the weights of male and female lobsters in relation to their lengths are compared for four areas, viz., Grand Manan (Seal cove), Canso, Pointe du Chêne and a mixture obtained from Pointe du Chêne, northern New Brunswick

and western Prince Edward Island. Among lobsters from each of these areas the same trend is apparent: while at the smaller sizes there is no significant difference between the weights of males and females, at larger sizes the weight of the male becomes greater than that of the female. At Grand Manan the difference

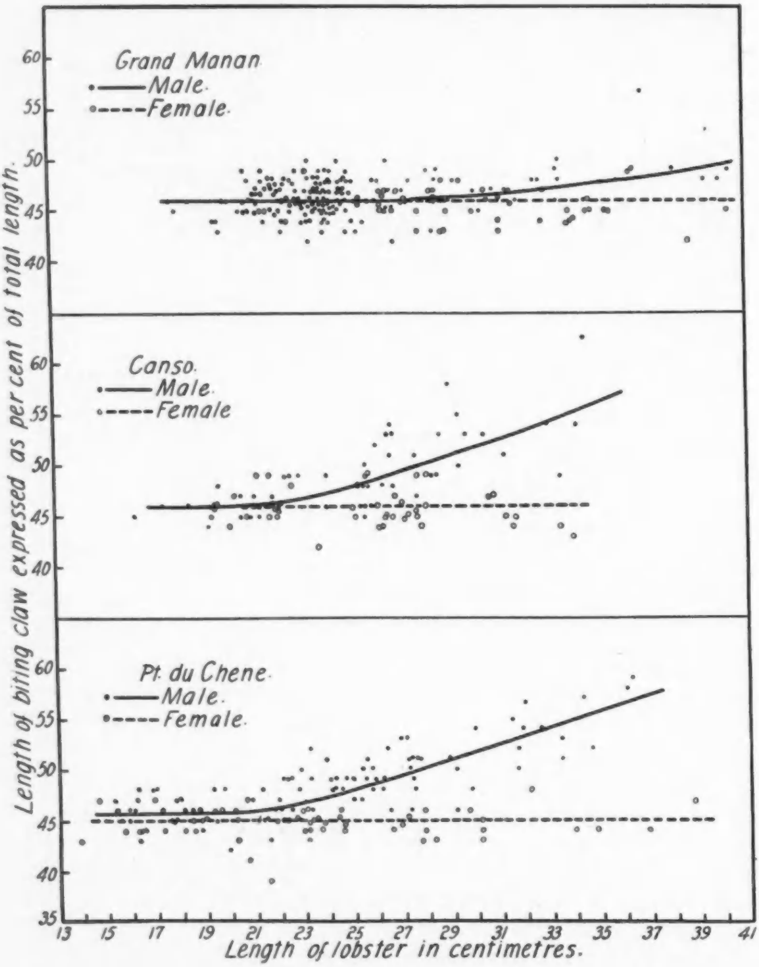


FIGURE 3. Increase in relative length of biting claw with increase in total length of male and female lobsters at Grand Manan, Canso and Pointe du Chêne, 1932.

between the weights of the male and female is much less significant than in the other regions compared. For example, while at Grand Manan male lobsters of 30 cm. are about three ounces (90 grams) heavier than the females, at Canso males

of this length are about nine ounces (250 grams) and at Pointe du Chêne about ten ounces (280 grams) heavier.

REGIONAL DIFFERENCES

Figure 6 gives a comparison of the relative length of the biting claw of male lobsters at Pointe du Chêne, with that at Grand Manan, cape Tormentine and Canso, and figure 7 the relative length of the crushing claw of male lobsters at

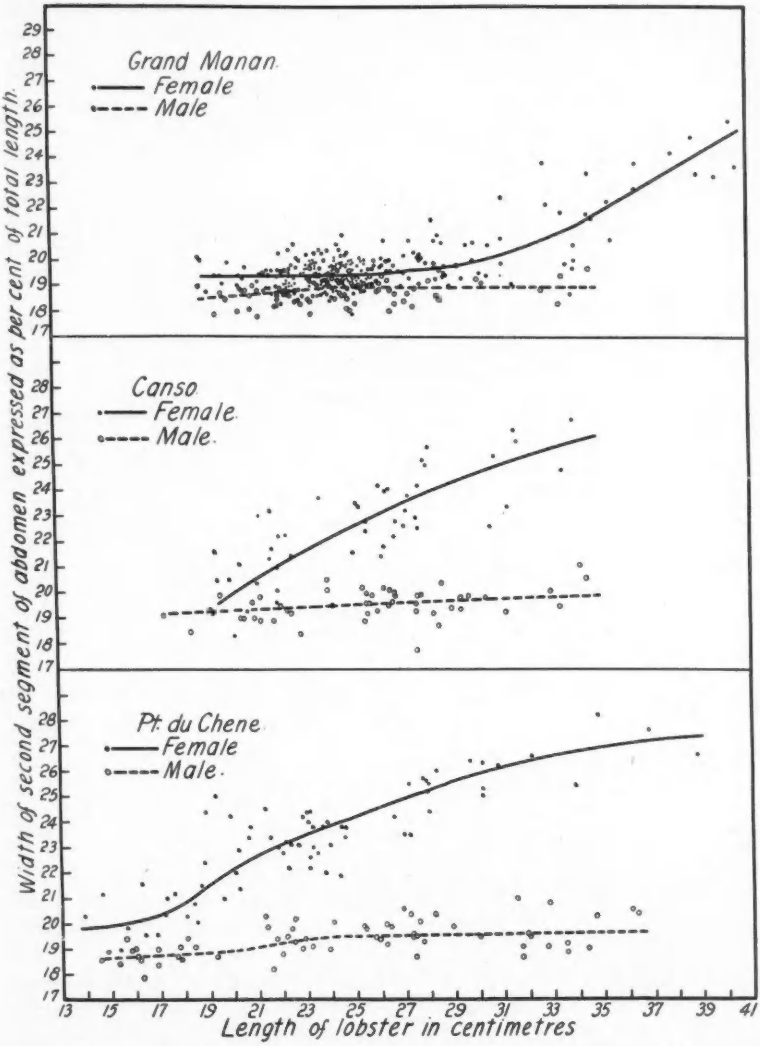


FIGURE 4. Increase in relative width of the second segment of the abdomen with increase in total length in male and female lobsters at Grand Manan, Canso and Pointe du Chêne, 1932.

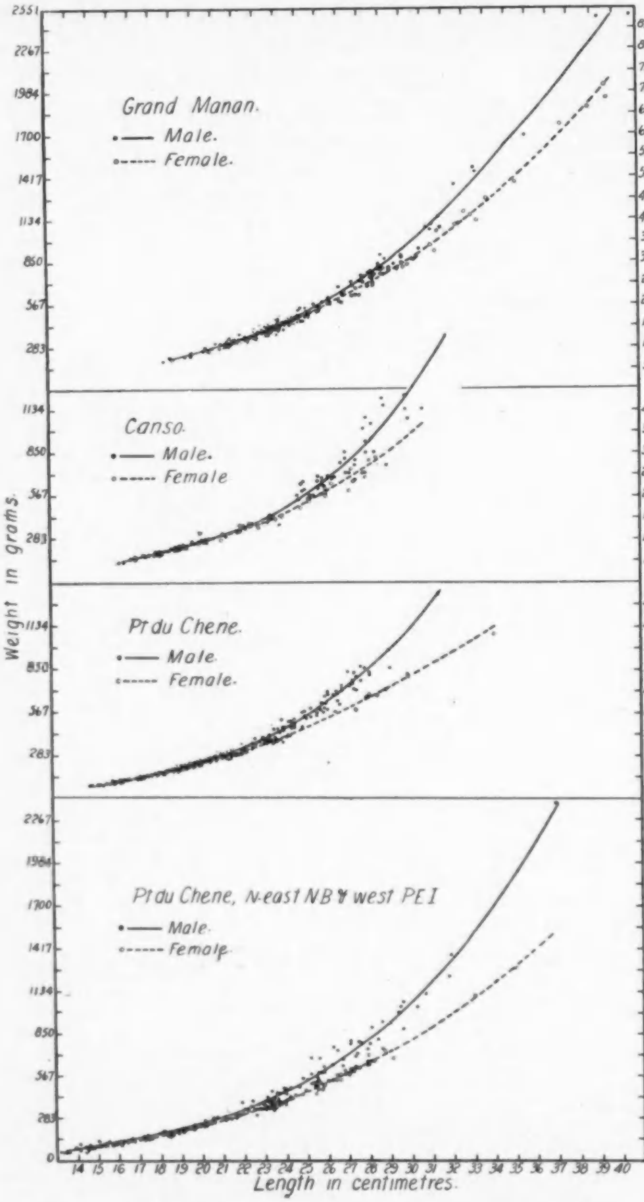


Figure 5. Increase in weight with increase in length of male and female lobsters at Grand Manan, Canso, Pointe du Chêne, and a mixture from Pointe du Chêne, northeastern New Brunswick and western Prince Edward Island, 1932.

Pointe du Chêne, Grand Manan and Canso. The relative length of the claws of the male is similar at both Grand Manan and Pointe du Chêne until the lobster is 20 to 22 cm. long, when the relative length of the claw increases at Pointe du

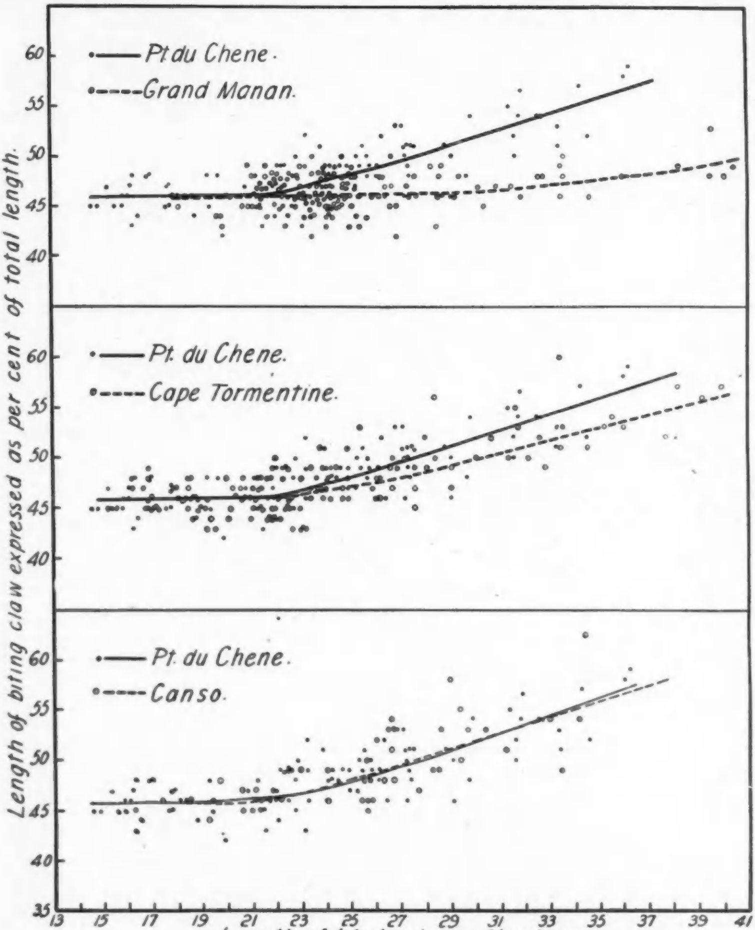


FIGURE 6. Increase in relative length of biting claw with increase in total length of the male lobster at Pointe du Chêne, Grand Manan, cape Tormentine and Canso, 1932.

Chêne while it remains constant at Grand Manan. Thus, the relative length of the claws of the male at all larger sizes becomes much greater at Pointe du Chêne than at Grand Manan. After the lobster at Grand Manan reaches a size of 30 to 33 cm. the relative length of the claws becomes greater, but even at the largest

sizes obtained does not approach the length of claw at Pointe du Chêne. Owing to intensive fishing no very large lobsters could be obtained but since the relative size of the claw at Pointe du Chêne is continually increasing toward the larger sizes, it is probable that even among the largest lobsters the relative size of the claws at Grand Manan is considerably smaller than at Pointe du Chêne. The relative size of the claw in the male lobsters at Canso and at cape Tormentine is quite similar to that at Pointe du Chêne, but more data would be required to

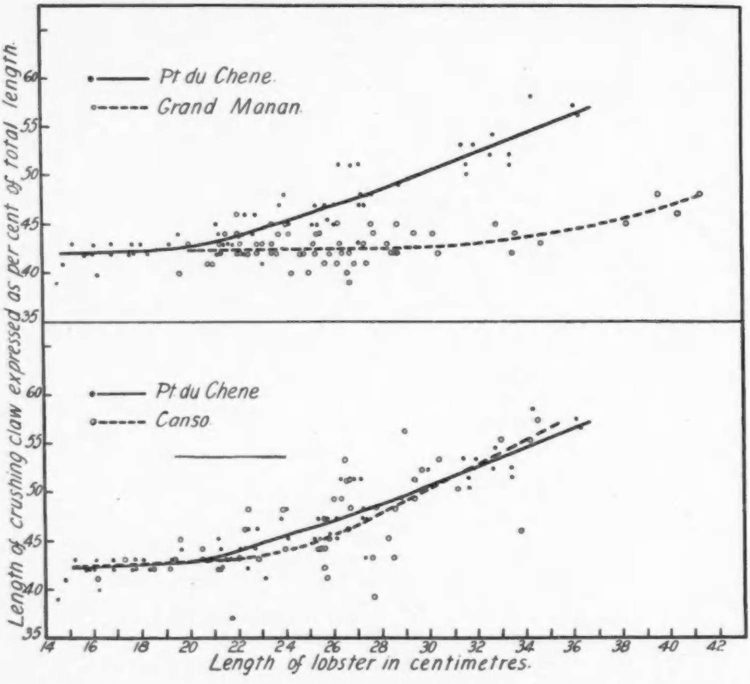


FIGURE 7. Increase in relative length of the crushing claw with increase in total length of the male lobster at Pointe du Chêne, Grand Manan and Canso, 1932.

determine exactly whether there is a significant difference between the relative sizes of the claws in these areas.

Figure 8 compares the relative width of the second segment of the abdomen of female lobsters at Pointe du Chêne with that at Grand Manan, Canso, and cape Tormentine. The absence of the smaller lobsters from Grand Manan catches makes impossible an exact comparison of the relative width of the second segment of the abdomen at these sizes at Pointe du Chêne and Grand Manan, but it seems probable that below 18 cm. in the two regions there is no very significant difference. However, at a total length of 18 and 19 cm. a sharp increase occurs in the relative width at Pointe du Chêne, and from this point onward the relative

width of the abdomen of the female at Pointe du Çhêne becomes considerably greater than at Grand Manan. The increase in the relative width of the second segment of the abdomen of the female at Grand Manan does not begin until the lobster has reached a size of about 33 cm., but beyond this point a sharp increase

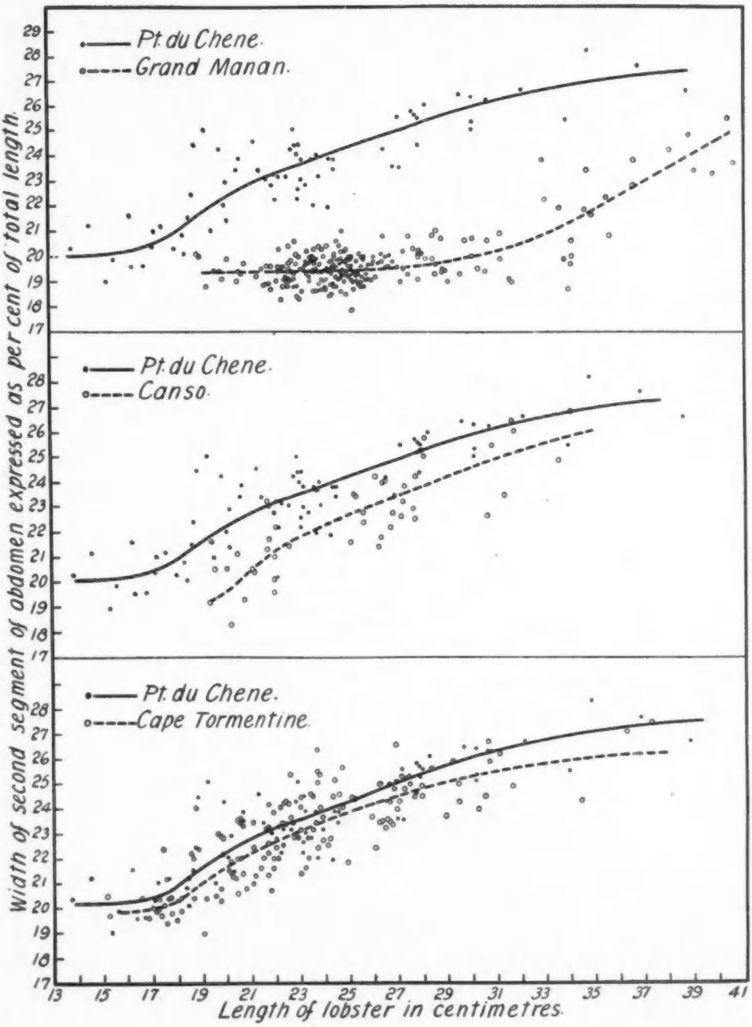


FIGURE 8. Increase in relative width of the second segment of the abdomen with increase in total length of the female lobster at Pointe du Chêne, Grand Manan, Canso and cape Tormentine, 1932.

occurs, although the relative width of the abdomen in even the largest specimens obtainable was considerably greater at Pointe du Chêne than at Grand Manan. At both Canso and cape Tormentine the width of the second segment of the abdomen is at all sizes somewhat similar to that at Pointe du Chêne. At Canso,

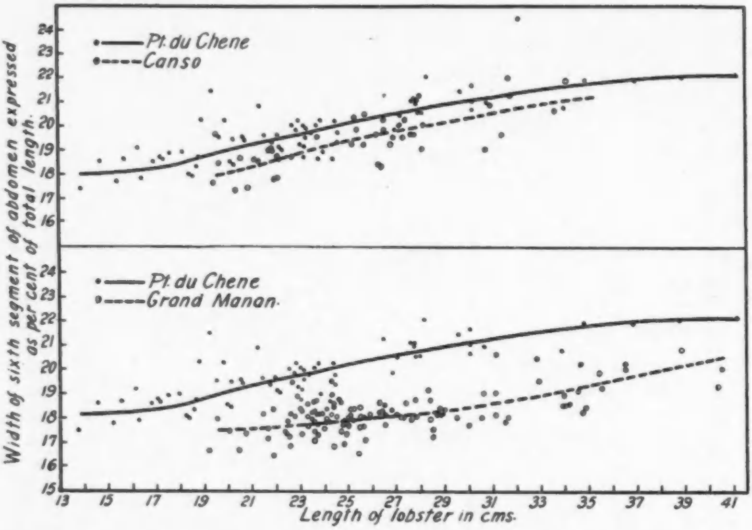


FIGURE 9. Increase in relative width of the sixth segment of the abdomen with increase in total length of the female lobster at Pointe du Chêne, Canso and Grand Manan, 1932.

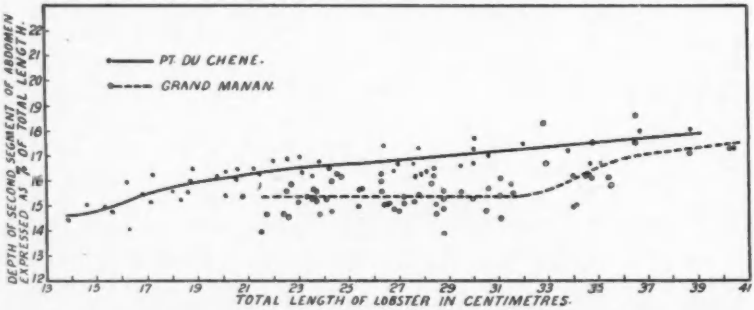


FIGURE 10. Increase in relative depth of the second segment of the abdomen with increase in total length of the female lobster at Pointe du Chêne and Grand Manan.

however, the increase in relative width occurs at a length several centimetres greater than at Pointe du Chêne and the relative width of the abdomen in the larger females is slightly smaller than at Pointe du Chêne. More data would be

required to determine the significance of the small differences shown in the width of the second segment of the abdomen at Pointe du Chêne and cape Tormentine.

Figure 9, comparing the relative width of the sixth segment of the abdomen of the female lobster at Pointe du Chêne with that at Canso and at Grand Manan, is in all essentials similar to figure 8 already discussed above, with, at the larger

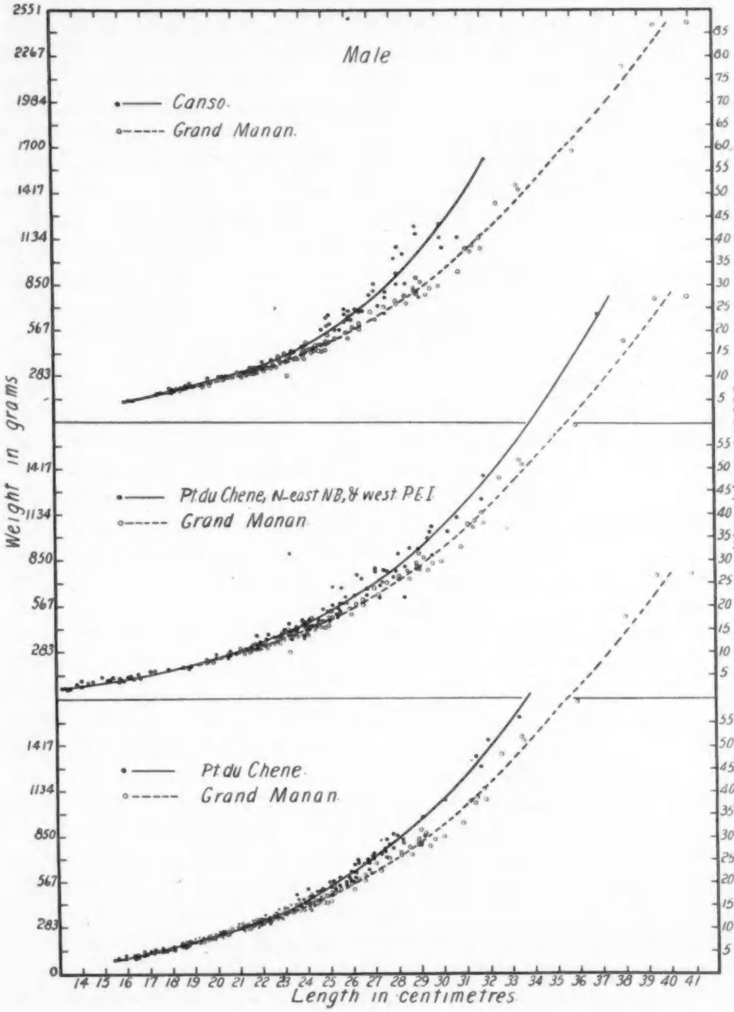


FIGURE 11. Increase in weight with increase in total length of the male lobster at Canso, Grand Manan, Pointe du Chêne, northeastern New Brunswick and western Prince Edward Island, and Pointe du Chêne, 1932.

sizes, a slightly greater width of the sixth segment of the abdomen of the female at Pointe du Chêne than at Canso and a much greater width at Pointe du Chêne than at Grand Manan. The increase in relative width of the sixth segment of the abdomen at the larger sizes is, however, not nearly as rapid as in the case of the second segment.

Figure 10 gives a comparison of the relative depth of the second segment of the abdomen of the female lobster at Pointe du Chêne and at Grand Manan. There is an increase in the relative depth of the abdomen of the female, beginning at about 16 to 17 cm. at Pointe du Chêne and about 33 cm. at Grand Manan.

Figure 11 compares the weights of old-shelled male lobsters at Grand Manan with those: (1) at Canso, and (2) at Pointe du Chêne, northeastern New Brunswick, and western Prince Edward Island, and also (3) with those of new-shelled hard-shelled lobsters at Pointe du Chêne. In all these latter areas the larger male lobsters are considerably heavier than at Grand Manan, e.g. with lobsters 32 cm. long the weight of the male in the other areas shown in figure 11 ranges from 7.1 to 15.9 ounces (200 to 450 grams) greater than at Grand Manan, with still greater differences at the larger sizes.

DISCUSSION AND CONCLUSIONS

Many workers on decapods have found in various species an increase in the relative width of the abdomen of the female, and in the relative size of the large claw (when present) of the male, beginning at sexual maturity. This condition exists in the lobster. At Pointe du Chêne the smallest egg-bearing female found was 18.0 cm. and the increase in the relative width of the abdomen of the female begins between 18 and 19 cm. At this size in the Pointe du Chêne area the width of the second segment of the abdomen of the female ranges from 20 to 25 per cent of the total length, a range of about 5 per cent as compared with a previous range of only about 2 per cent (see figure 8). The lower relative widths at 18 to 19 cm. apparently represent immature females, while the higher relative widths represent the smallest mature females. At larger sizes the difference between the highest and lowest relative widths becomes less, all the lobsters being apparently mature. At cape Tormentine and Canso the smallest egg-bearing females found were several centimetres larger than at Pointe du Chêne. The true difference in the size of the smallest egg-bearing females in these areas may be even less since the cape Tormentine and Canso feeding grounds were not so thoroughly investigated as the Pointe du Chêne area. In accordance with this small difference in the size at which sexual maturity begins in these areas is very little difference in the relative width of the abdomen of the mature females at Pointe du Chêne and at cape Tormentine and Canso. At Grand Manan the smallest egg-bearing lobster found was 34.6 cm. in length, and corresponding with this very large size at first sexual maturity it is only at 33 to 34 cm. that the increase in the relative width of the abdomen of the female occurs. At this size at Grand Manan (see figure 8) the width of the second segment of the abdomen ranges from 18.5 per cent to 23.8 per cent, a range of 5.3 per cent, although the range previous to this size was only 2 or 3 per cent. The lower relative widths at 33 to 34 cm. apparently represent

immature females and the higher, the smallest sexually mature females. At larger sizes the difference between the highest and the lowest relative widths becomes less, all the lobsters being apparently mature.

While the smallest sexually mature sizes in the female lobster were easily determined from the sizes carrying eggs, fewer data are available on the sizes of the smallest sexually mature males. However, during experiments on lobster-mating at Pointe du Chêne in 1932, mating was successfully accomplished by a male of 20.5 cm. in length. Another male of 19.0 cm. attempted mating four or five times with a soft-shelled female of 23.0 cm., but did not succeed, and hard-shelled males of 17.5 and 18.3 cm. respectively made no attempt at mating with a female of 21.2 cm., which later mated with a male of 22.5 cm. From these records it seems probable that in the Pointe du Chêne area, where the smallest egg-bearing female obtained was 18.0 cm. in length, the size of the male at first sexual maturity is not greatly different from that of the female. Thus, while first sexual maturity, judged by the smallest egg-bearing females, occurs between 18 and 20 cm. at Pointe du Chêne, Canso, and cape Tormentine, in figures 7 and 8 the increase in relative length of the claw in the male begins between 21 and 22 cm., while at Grand Manan, with first sexual maturity of females about 34 cm., the increase in the relative length of the claws in the male begins at a total length of about 31 to 33 cm. or larger. The exact point of upturn in the Grand Manan graphs is rather indefinite owing to the scarcity of specimens of the larger sizes.

At Pointe du Chêne the increase in the relative width of the abdomen of the female occurs between 18 and 19 cm., while the increase in the relative length of the claw of the male begins between 21 and 22 cm. This may mean that sexual maturity occurs at a slightly larger size in the male than in the female in this area, or if sexual maturity is at the same size in both sexes, the approach to sexual maturity may be manifested more rapidly in the case of the abdomen of the female than with the claws of the male.

The greater weight of mature male lobsters than female, and of mature male lobsters at Pointe du Chêne, northern New Brunswick and western Prince Edward Island, and at Canso, than of those at Grand Manan is chiefly due to the larger size of the claws of the male than of the female, and to the larger size of the claws of the male in the above-mentioned regions than at Grand Manan.

The wide abdomen with deep pleura, giving a large protected ventral space, is useful to mature females since it provides a cavity of larger volume for containing and protecting the eggs. The usefulness of larger claws to the male is not at first sight so evident. In lobster-mating experiments at Pointe du Chêne in 1932, when two males were placed in the presence of a soft-shelled female, the males sometimes fought, the superior male mating with the female. Almost invariably after mating, the male which had mated would stand guard and attack the other males approaching the female. In fighting occurring between two lobsters with similar hardness of shell, it is the size of the big claws that largely determines superiority, and this particular function of the large claws connected as it is with the act of copulation, gives significance to the relation between sexual maturity and the increased size of the large claws of the male.

ACKNOWLEDGEMENT

The above investigations were carried out while the writer was engaged as scientific assistant of the Biological Board of Canada at the Atlantic Biological Station.

REFERENCES

HERRICK, F. H. *Bull. U.S. Bur. Fish.* **29**, 149-408, 1911.
HUXLEY, JULIAN S. Problems of relative growth. 1st ed. 276 pp. London, 1932.